

THIS REPORT HAS BEEN DELIMITED
AND CLEARED FOR PUBLIC RELEASE
UNDER DOD DIRECTIVE 5200.20 AND
NO RESTRICTIONS ARE IMPOSED UPON
ITS USE AND DISCLOSURE.

DISTRIBUTION STATEMENT A

APPROVED FOR PUBLIC RELEASE;
DISTRIBUTION UNLIMITED.

Armed Services Technical Information Agency

AD

44933

NOTICE: WHEN GOVERNMENT OR OTHER DRAWINGS, SPECIFICATIONS OR OTHER DATA ARE USED FOR ANY PURPOSE OTHER THAN IN CONNECTION WITH A DEFINITELY RELATED GOVERNMENT PROCUREMENT OPERATION, THE U. S. GOVERNMENT THEREBY INCURS NO RESPONSIBILITY, NOR ANY OBLIGATION WHATSOEVER; AND THE FACT THAT THE GOVERNMENT MAY HAVE FORMULATED, FURNISHED, OR IN ANY WAY SUPPLIED THE SAID DRAWINGS, SPECIFICATIONS, OR OTHER DATA IS NOT TO BE REGARDED BY IMPLICATION OR OTHERWISE AS IN ANY MANNER LICENSING THE HOLDER OR ANY OTHER PERSON OR CORPORATION, OR CONVEYING ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE OR SELL ANY PATENTED INVENTION THAT MAY IN ANY WAY BE RELATED THERETO.

Reproduced by
DOCUMENT SERVICE CENTER
KNOTT BUILDING, DAYTON, 2, OHIO

UNCLASSIFIED

AD NO. ~~44~~ 933
ASTIA FILE COPY

CALIFORNIA INSTITUTE OF TECHNOLOGY

WATKINS AND CRELLIN LABORATORIES OF CHEMISTRY

Pasadena, California

INFRARED INVESTIGATIONS
RELATING TO THE
STRUCTURE OF POLYPEPTIDES AND PROTEINS

Technical Report No. 8

and

Final Report

October 31, 1954

Office of Naval Research

Contract N6ori-102, Task Order VI

Project NR-055-019

NR-055-199, Contract N6ori-102, Task Order VI

CALIFORNIA INSTITUTE OF TECHNOLOGY

Gates and Crellin Laboratories of Chemistry

Pasadena, California

Infrared Investigations Relating to the
Structure of Polypeptides and Proteins

Technical Report No. 8

and Final Report

October 31, 1954

Project Supervisor: Richard M. Badger

Participants in Work: A. D. E. Pullin
Richard M. Badger
Hector Rubalcava

TABLE OF CONTENTS

	Page
Section 1. Introduction	1
Section 2. A Normal Coordinate Treatment of the Vibrations of the Peptide Group with the Use of a Mechanical Model . . .	3
Richard M. Badger	
Section 3. The Polarized Infrared Spectrum of α -Diglycine A. D. E. Pullin	
Part I. General Study	19
Part II. The Transition Moment Direction of the N-H Valence Vibration	37
Section 4. An Investigation of the Spectrum of Silk Fibroin	46
A. D. E. Pullin and Richard M. Badger	
Section 5. Summary of Information Relating to the Direction of Change of Dipole Moment in the Characteristic Amide and Peptide Vibrations	55
Section 6. The Measurement of Infrared Extinction Coefficients and Dichroic Ratios of Solids	63
A. D. E. Pullin	
Section 7. Structure in the 3μ Region of the Spectra of Associated Amides and Carboxylic Acids	70
Richard M. Badger and Hector Rubalcava	
Section 8. The Infrared Spectrum and Structure of Collagen Richard M. Badger and A. D. E. Pullin Reprinted from the Journal of Chemical Physics, <u>22</u> , 1142 (1954).	
Section 9. The Infrared Association Spectra of Amides in Solution and their Relation to the Spectra of Polypeptides Richard M. Badger and Hector Rubalcava Reprinted from the Proceedings of the National Academy of Sciences, <u>40</u> , 12 (1954).	

INTRODUCTION

Though it seems certain that in the final and complete elucidation of protein structures the use of x-ray methods will play a prominent role, the determination of even the simplest such structure with certainty in respect to all details will probably require the combined assistance of all available methods of investigation, both chemical and physical, and these methods will have to be extended and improved.

It has already been adequately shown that infrared spectroscopy is an invaluable tool in the investigation of polypeptides and proteins and is likely to be of continually increasing utility as our knowledge of the relation between structure and spectrum develops. The applications so far made have been largely qualitative in nature. Though it is quite evident that these have not been by any means fully exploited it is probable that the value of spectroscopy could be very considerably increased by more quantitative applications.

The present report deals in part with qualitative observations chiefly relating to the identification of characteristic absorption bands in amides and peptides, but to a greater extent describes some preliminary attempts to make more quantitative use of intensity measurements in the elucidation of composition and structure. Section 10, for example, describes a quantitative study which resulted in a reliable estimate for the energy of the N-H...O hydrogen bond and gave information relating to the structures of amide polymers in solution. Sections 2, 3, and 4 relate largely to another possible use of quantitative intensity measurements.

We have recently been interested in the possibility that a quantitative measurement of dichroism could be of value in the investigation of certain structural details. For this approach to be really effective it is probable that considerable advances will have to be made in two directions. First of all some improvement will be required in instrumentation, so as to permit precise intensity measurements to be made with really adequate resolution under rather difficult conditions. Secondly, the techniques of preparation of samples of material for investigation require further development, and reliable criteria for estimating the degree of orientation of the samples and the extent of crystallinity need to be worked out. Nevertheless it seemed desirable to explore the possibilities of the method with means at present available and to discover the obstacles which may lie in one's way.

An approach to the problem has been made in three ways. In the first place we have made an attempt to understand the normal vibrational modes which give rise to the characteristic amide and peptide infrared absorptions, and to find the cause of some observations regarding intensity and frequency which appear anomalous when the amides are considered as a whole, (Section 2 of this report.) An objective of this study was to develop a qualitative notion as to the probable direction of change of electric moment in the peptide vibrations and to explain some experimental observations in this regard. Secondly, we have collected experimental information regarding this change in dipole moment and the possible variability of direction from one compound to another both by investigations of simple crystalline substances, (Section 3 of this report and Technical Reports No. 6 and 7) and by the use of data on polypeptides for which probable structures, supported by x-ray studies, have been proposed, (Sections 4 and 5.)

A NORMAL COORDINATE TREATMENT OF THE VIBRATIONS
OF THE PEPTIDE GROUP WITH THE USE OF A MECHANICAL MODEL

By Richard M. Badger

An important step in the understanding of the vibrational modes of the trans amide group was recently made by Price and Fraser¹ who suggested that the three characteristic vibrations in the range 1000 - 1700 cm^{-1} can approximately be regarded as follows: the 1650 cm^{-1} frequency as a pseudo asymmetric or ν_3 vibration of an O=C-N non linear triatomic group, and the 1550 and 1250 cm^{-1} frequencies as pseudo symmetric vibrations of the same group, coupled in two ways with an N-H bend. The agreement on this interpretation is possibly not universal, and some anomalies remain to be explained. Less progress has been made in the complete understanding of the unsubstituted amide, of the trans amide group, and of the N,N-doubly substituted amide.

It is probable that a complete normal coordinate treatment of the various amides (including unsubstituted, singly N-substituted in cis and trans configurations, and doubly N-substituted forms) and their deuterium substituted derivatives would be very useful. This, however, would be a rather formidable undertaking since at least nine different models need to be considered, which have no element of symmetry except the plane in which the atoms lie. The minimum model which should be expected to meet the requirements is hexatomic. This is adequate for the first rough approximation but is not sufficient to account for some very important details. In particular this model cannot be expected to indicate and reason for the shift in frequencies in going from the α to the β configuration in polypeptides.

It occurred to us that a preliminary attack on this problem could readily be made by the use of vibrating mechanical models which would give a good representation of the character of the normal modes even though it might be difficult to represent the relative frequencies precisely. Such models should furthermore be of assistance in helping to decide the further elaboration which will be necessary to represent the finer details of the experimental observations. We should like to point out that in mathematical normal coordinate treatments the amplitudes in the normal modes have very seldom been calculated. In unsymmetrical molecules these would seem to be quite as important as the frequencies and a normal coordinate treatment cannot be said to be complete until intensities and directions of polarization have been at least qualitatively accounted for.

Several attempts have been made in the past to investigate the normal vibrations of simple molecules by the use of mechanical models simulating these molecules.² The general problem of a three dimensional structure, however, presents almost insuperable difficulties. The planar amide group, on the other hand, in which the in planar vibrations are of chief interest, is an ideal case for this method of attack.

Description of the Mechanical Model

A vibrating model has consequently been constructed which differs in several respects from those previously employed. In particular, weights of appropriate relative mass representing the atoms are suspended in a plane by fine nichrome wires 0.005 in diameter and about two meters in length. In the range of interest the restoring force due to the pendulum effect is negligible and this method of support permits the use of very light springs to represent the bonds between atoms. The mass of these springs is small even in comparison with a hydrogen atom, which was not true in some earlier models. The frequen-

cies are consequently in a convenient range for the direct observation of the normal modes; a molecular frequency of 1500 cm^{-1} is represented by a model frequency of about 4 cycles per second. The damping is small and without excitation a molecular vibration will persist for about 100 cycles with sufficient amplitude for observation.

The atomic masses are represented by bronze balls weighing 5.23 gm per atomic weight unit. Since only two sizes, 1 inch and $7/16$ inch in diameter, representing carbon and hydrogen atoms, were readily available, auxiliary lead weights were attached in the case of heavier atoms, or for studying the effect of isotopic substitution. All heavier atoms were provided with a small soft steel armature attached to the bottom. Around the equator of each ball, sockets with set screws were provided, spaced 120° apart for the attachment of springs.

Since we had found that helical springs are likely to have vibrational modes of their own in the range of interest, which may produce confusing effects, another type of spring was adopted. As may be seen in Figure 1 it consists of a single circular loop of steel spring wire, usually 0.016 inch or 0.018 inch in diameter with projections provided diametrically for attachment to the atoms. These springs are quick and simple to fabricate and are easily reproducible. They provide automatically about the correct ratio of stretching and bending force constants, though this is adjustable by changing the length of the projections for attachment. The mass of a spring ranges from 0.48 gms for a CH bond to less than twice this amount for a CO double bond. It should be mentioned at this point that the model is driven by a number of solenoids wired in parallel located below the atoms and slightly displaced from the rest positions in appropriate directions. The solenoids are activated by a vibrating reed of which the length is continuously variable to provide for tuning. The sharp tuning of the model is both an advantage

and an inconvenience, since the location of all the normal modes is somewhat time consuming when their character and frequency is not known.

One of the models is shown in Figure 1, which represents N-methyl acetamide stripped of the hydrogen atoms of the methyl groups. Anything less than a hexatomic model is rather inadequate, but this model is sufficient to represent all except the finer details of the planar vibrations of the peptide link. The preliminary choice of relative force constants was guided by the Pauling internuclear distances for the peptide link.³ Adjustments were then made as we found necessary.

It will be noted in the figure that oxygen and nitrogen atoms are connected by a supplementary helical spring which though not quite satisfactory represents the best simple approximation we could find for a bond-bond interaction constant. A large interaction constant of this sort is to be expected in any triatomic group where two resonating structures contribute to the ground state, as is indicated by the anomalous distances in the amide group. A very similar situation exists in ozone and NO_2 ⁴ as will be mentioned later. We soon found that such an interaction constant seems to be required in order to bring the amide frequencies at 1540 and 1650 cm^{-1} into the close proximity in which they exist.

Relative Frequencies Predicted by the Models

We shall be most interested in the character of the normal vibrations of the various models, but first it will be of interest to see how well the model frequencies correlate with observation for the case of the N-substituted trans acetamide and to consider the predictions made in other cases.

The second section of Figure 2 compares the three amide frequencies in the range 1000 - 1800 cm^{-1} given by the present N-methyl acetamide model with those observed frequencies with which we believe they should be correlated,

together with the shifted frequencies resulting from N-deuteration. Qualitatively the model seems satisfactory in locating three frequencies in the correct position to better than 3 per cent, and in predicting the relative magnitudes of the isotope shifts. (The scale factor relating observed and model frequencies is arbitrary of course.) The more serious defect seems to be in the prediction of a too large isotope shift of the lowest frequency. The reason for this will become obvious when we consider the normal modes. It can easily be corrected by increasing the strength of spring between nitrogen and N-methyl carbon to bring a C-N single bond frequency up from 870 into the neighborhood of 1000 cm^{-1} which is its more probable location. A very slightly larger N-H bending constant would also result in improvement.

As will be shown later, the normal modes of this model are indeed of the character predicted by Price and Fraser. Indeed the actual motions are such that the terms often used: carbonyl, C-N and N-H bend vibrations, are very little descriptive, and it seems preferable to extend the nomenclature of Randall et al.⁵ We shall in all cases call the highest frequency member of the group Amide I, and the other two Amide IIa and Amide IIb in order of decreasing frequency.

We will now consider three other models constructed with precisely the same force constants as before, of which the first represents the unsubstituted acetamide. As is shown in the top section of Figure 2 all the model frequencies are significantly higher. This is easy to understand in the case of Amide IIa and IIb, since the NH_2 scissors frequency will be expected to be higher than a N-H bend. For example in chloramine the NH_2 scissors is at 1553 cm^{-1} while in dichloramine the N-H bend is at 1295 cm^{-1} .⁶ In the simple unsubstituted amides a characteristic band is always found between 1580 cm^{-1} and 1600 cm^{-1} which is characterized by the increase in frequency which results from hydrogen bonding. We designate this as Amide I, and our

model shows that the normal mode is largely a NH_2 scissors bend. It may have some significance to say that in the unsubstituted amide the N-H frequency has crossed over the pseudo symmetrical triatomic stretch. It will be shown later that in the Amide I vibration there is considerable coupling with the NH_2 scissors, for which there is no analogue in the N-substituted amide. This serves to push Amide I to significantly higher frequencies, in agreement with our experimental observations on a series of amides.

The identification of Amide IIb in acetamide is somewhat less positive though a band in the position indicated in Figure 2 is common to the unsubstituted amides. In formamide a quite certain identification can be made. In a moderate path in the vapor only one band of significant intensity is seen in this region, namely at about 1256 cm^{-1} . In the hydrogen bonded state, as for example in the liquid, this band shifts up to 1303 cm^{-1} and is rather broad. In the liquid state this band is accompanied by a narrow and intense satellite at 1395 cm^{-1} which is undoubtedly the CH bend activated by interaction with Amide IIb. The unperturbed IIb would therefore lie somewhere between 1303 and 1350 cm^{-1} . This is an interesting example of changes in intensity due to interaction which are sometimes so difficult to interpret in deuteration experiments.

Evidently some improvement in the model would result from a slight increase in the NH bending force constant as was noted in the previous model.

The cis amide model is of particular interest since the question has several times arisen as to whether this configuration exists in proteins.^{7,8} The only compounds in which the cis amide arrangement is definitely known to exist are those in which it exists of necessity because of a cyclic structure, as is found in diketopiperazine and the lactams. Cyanuric and parabanic acids represent rather special cases, and certainly cannot be regarded as typical. In general these compounds are characterized by a rather high Amide I frequency,

which is, however, dependent on ring size in the lactams,⁹ and an absence of significant absorption in the 1500 - 1600 cm^{-1} region (Amide IIa.) In most cases no positive identification has been made of an isotope shift in the region 1100 - 1600 cm^{-1} . In diketopiperazine 1340 cm^{-1} is probably to be identified as Amide IIb since it disappears on deuteration though the shifted frequency was not picked up. In cyanuric acid the situation is quite clear but the model is rather too special to warrant discussion in this connection. The unusually high frequency of Amide IIb in both diketopiperazine and cyanuric acid in the crystalline state probably results from the very strong hydrogen bonding in symmetrical eight membered rings. This was not allowed for in our model.

Our model constructed with the same force constants used previously predicts rather high Amide I and low Amide IIb frequencies. This seems to be caused by a large interaction between the asymmetric triatomic mode and the N-H bend resulting from the new geometrical arrangement. There is a great alteration in the character of Amide IIa, which contains a smaller contribution of N-H bend than in the trans model, accounting for its lower frequency. The inactivity of Amide IIa in the actual molecules will be discussed below.

The model of the N,N-disubstituted amide (Figure 2, Section 4), has much in common with the cis amide model. Amide I is somewhat high, in agreement with experiment, and Amide II is low because of the absence of any contribution of N-H bend.

Our model was constructed with the same force constants as the preceding ones though we have at present no assurance that they should be quite the same, since we know of no structure of this sort which has been examined by x-ray methods. It is possible that the C-O bond constant should be somewhat stronger and the amide C-N bond somewhat weaker owing to the absence of the

intermolecular hydrogen bonding which in the previous cases appears to have an important effect on these linkages. In the observed spectra strong absorption is again absent in the region $1500 - 1600 \text{ cm}^{-1}$. In several doubly substituted amides a weak absorption is present near 1500 cm^{-1} . (In diethylacetamide at 1495 cm^{-1} and in dimethyl formamide at 1485 cm^{-1} .) This seems rather high for a methyl frequency and we tentatively correlate it with the frequency predicted by the model. We do not agree with the suggestion of Letaw and Gropp¹⁰ that Amide II has moved up and fused with Amide I, since measurements made in this laboratory by Mr. Rubalcava have shown that the intensity of Amide I is closely the same in N,N-dimethyl formamide as in the singly N-substituted compound.

Now the primary purpose of the model treatment was to examine the character of the normal modes and to assist us in drawing some conclusions regarding the associated intensities of absorption and directions of polarization. In particular it would be very desirable if it were possible to give answers to the following questions:

- a) Why is Amide IIa in the normal trans amide so little shifted by deuteration though its intensity is frequently greatly decreased?
- b) Why is Amide IIa absorption essentially missing from the spectra of the cis amide and the N,N-disubstituted compounds?
- c) What is the explanation of a band at 3080 cm^{-1} which appears in the spectra of the associated, monosubstituted amides and of polypeptides, and which has been regarded as exhibiting anomalous polarization behavior?

Before discussing the normal modes it will be convenient to make one general remark regarding intensities. The Amide I band is very intense and in general is the one outstanding band in the spectra of polypeptides. This appears perfectly reasonable in view of the amide structure and the character of the normal mode. From the internuclear distances we may presume that two

electronic structures make significant contributions to the amide ground state. In one of these the oxygen and nitrogen atoms bear positive and negative formal charges; in the other they do not. During the unsymmetrical vibration one may expect the relative contributions of the two structures to vary greatly, as shown in Figure 3, resulting in a large change in charge distribution and a large change in dipole moment. In the symmetrical vibration if the CO and CN

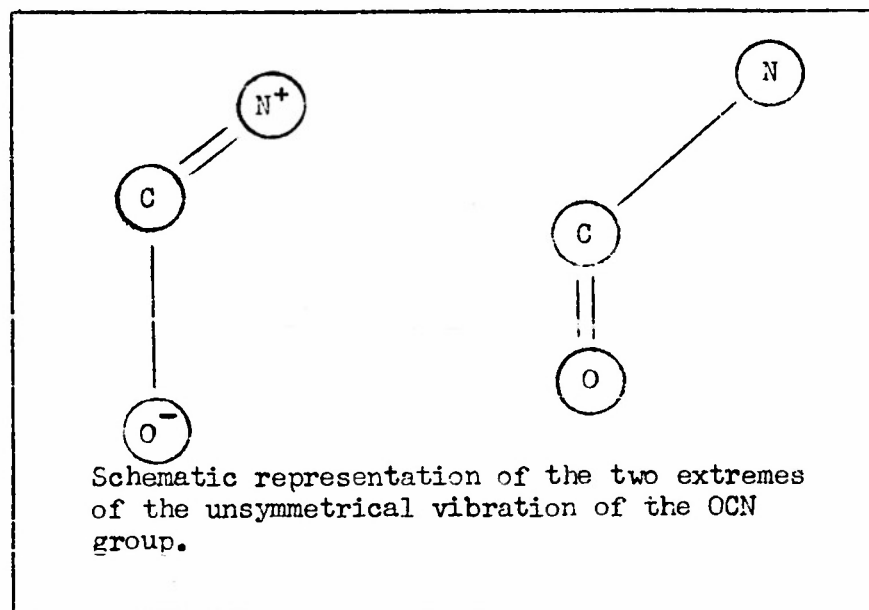


Figure 3

distances both change fractionally by about the same amount the change in dipole moment may well be very much smaller.

A similar situation is found in ozone and NO_2 where M. Kent Wilson showed that the ratio of intensities of ν_3 to ν_1 are of the order of 100/1 and 50/1, respectively.⁴

The Amide IIa band is generally less intense than Amide I but is nevertheless of considerable intensity. Let us assume tentatively that this results largely from the contribution of the N-H bend. As will be pointed out this provides an explanation for several facts which have appeared anomalous.

The Normal Modes of the Amide Models

In figures 4, 5, 6, 7 and 8 which follow the conclusion of this section a graphical representation is given of the more important normal modes of several of the mechanical amide models investigated. These modes in general include only those which lie in the frequency range $1100 - 1800 \text{ cm}^{-1}$, namely Amide I, Amide IIa and Amide IIb, though in N-methyl formamide a C-H bend is shown which interacts considerably with one of the above modes. No attempt has been made to represent the hydrogen stretching vibrations which interact relatively little with the other amide modes. A number of the low frequency bending modes have been investigated, but they have not been presented since in general they will be rather dependent on the complexity of the model and will not be a constant property of the peptide group.

All models were hexatomic and they differ only in the number and arrangement of hydrogen atoms. The particular model used in each case should be obvious from the diagram. The direction of motion and amplitude of each atom is represented by a vector drawn through its center. Vectors are not shown for atoms having essentially negligible amplitudes. No attempt has been made to normalize the normal modes; rather the amplitude of the central carbon atom has been made the same in all cases where this was practical. In the "deuterated" models the deuterium atoms are represented by solid black circles and the modes of "protonated" and "deuterated" models are given side by side for comparison.

The models represented are enumerated in the following section where a discussion is given of the normal modes.

Discussion of the Normal Modes

1. (a) The unsubstituted amide (essentially acetamide stripped of the methyl hydrogen atoms).
- (b) The N,N-deuterated model.

It will be noted that in Amide I there is a large contribution of the NH_2 scissors bend which tends to keep the frequency rather high. This is greatly reduced in the deuterated model though the frequency shift on deuteration is not very great in our model.

The higher Amide II frequency does not show the pseudo symmetric vibration of the three central atoms proposed by Price and Fraser, though this is indeed present in the lower frequency of the pair. This is just the reverse of the situation found in the singly N-substituted model. It may have some significance to say that the N-H bend lies on opposite sides of the symmetric stretch in the two models.

2. (a) The singly N-substituted amide trans form (essentially acetamide stripped of all methyl hydrogen atoms).
- (b) The N-deuterated trans model.

This model clearly presents the situation regarding Amide I and Amide IIa which was predicted by Price and Fraser.¹ Amide IIb is not so simply described and changes significantly in character on deuteration, when the two methyl carbon atoms begin to participate considerably in the vibration. It is evident that the frequency shift of IIb depends on the C-C and C-N single bonds involved. Clearly the directions of change of dipole moment may shift considerably on deuteration. In IIa the amplitude of the N-D bend is relatively small which may reduce the intensity if this is to be attributed in considerable part

to the N-H bend in the normal molecule.

3. (a) The singly N-substituted amide, cis form. (This might represent a portion of a lactam or of diketopiperazine.)

(b) The N-deuterated cis model.

The change from trans to cis forms materially alters the normal modes. The N-H bend contributes significantly to I and tends to raise its frequency, but should have little effect on the direction of change of dipole moment though it might increase the intensity. The character of Amide IIa seems to provide an explanation for the low intensity associated with this mode. The effect of the hydrogen bend will to a considerable extent neutralize the dipole produced by the motion of the heavy atoms.

4. The N,N-disubstituted amide (representing N,N-dimethyl acetamide stripped of all hydrogen atoms).

In regard to the motions of the three central atoms, both amide vibrations preserve the simple character seen in Model No. 2, but the hydrogen bend is of course absent. Unless the contribution of this bend is largely responsible for the intensity of Amide II in the singly substituted molecule there seems to be no obvious reason why there should not be an equally intense and characteristic band in the spectrum of the disubstituted amide. The fact that no such band seems to be observed appears to support the belief that the change in dipole moment resulting from the motion of the heavy atoms is rather small.

5. (a) N-methyl formamide (stripped of methyl hydrogens).

(b) The N-deuterated model.

This model shows an example of interaction of another mode with the amide vibrations. In Amide I the participation of the C-H bend is very significant, tending to raise the frequency. In the other modes this effect is insignificant for geometrical reasons, although the C-H bend lies only slightly below Amide IIb.

Conclusion

We believe that the normal coordinate investigation made with the mechanical model has resulted in plausible explanations for several of the major features of the amide spectra both as regards frequency shifts and intensities. The deuteration shifts can be accounted for and a possible explanation for the inactivity of Amide IIa in the cis-amide and doubly N-substituted model has been presented. In this connection the band found near 3080 cm^{-1} in the associated singly N-substituted amides and in many polypeptides may be commented upon. Elsewhere in this report reasons are given for assigning this band to the overtone of Amide IIa. The polarization of this band in oriented materials is, however, frequently the same as that of the N-H stretching fundamental which might seem anomalous unless the intensity of Amide IIa is primarily due to the N-H bend. In this case the polarization mentioned is not unexpected. However, since Amide IIa involves both pseudosymmetric stretch and N-H bend, one might expect it to be somewhat sensitive to environment, both in regard to intensity and polarization. As is shown elsewhere this appears actually to be the case. Amide I, on the other hand is usually a simpler vibration and the high intensity results from a large, first order effect. It may consequently be somewhat less sensitive to environment.

Our treatment may perhaps be a starting point from which one may proceed to explain several important details which are still rather obscure. For this purpose it may be advantageous to elaborate somewhat on the existing model. A point of particular interest is the difference in frequency of the amide modes in polypeptides having α and β structures. It is unlikely that this results entirely from a difference in the hydrogen bonding situations since

all frequencies are shifted in the same direction. It seems more probable that it is a result of interactions with adjacent groups, which are different in the two configurations. In the future it is probable that it will be worth while to pay more attention to lower frequencies including both single bond stretches and the higher frequency bending modes, since it is probable that two of these are involved in a band at $1688-1696\text{ cm}^{-1}$ which appears in polypeptides with a high glycine content. This is discussed in the section on a diglycine.

References for Section 2

1. W. C. Price and R. D. B. Fraser, Proc. Roy Soc. (London) B, 141, 1 (1953).
2. See G. Herzberg, Infrared and Raman Spectra of Polyatomic Molecules,
D. Van Nostrand Company, Inc., New York, 1945, page 157 for references.
3. R. B. Corey and L. Pauling, Proc. Roy. Soc. (London) B, 141, 10 (1953).
4. M. Kent Wilson, Ph.D. Thesis, California Institute of Technology, 1948.
5. Randall, Fowler, Fuson and Dangel, Infrared Determination of Organic Structures. D. Van Nostrand Company, Inc., New York, 1949.
6. G. E. Moore and R. M. Badger, J. Am. Chem. Soc., 74, 6076 (1952).
7. L. Pauling, R. B. Corey, and H. R. Branson, Proc. Nat. Acad. Sci.,
(U. S.) 37, 272 (1954).
8. F. H. C. Crick, J. Chem. Phys., 22, 347 (1954).
9. Heinrich B. Kessler, Ph.D. Thesis, University of Michigan, 1952.
10. H. Letaw and A. H. Gropp, J. Chem. Phys. 21, 1621 (1953).

Captions for Figures of Section 2.

Fig. 1. Diagram of a mechanical model used in the normal coordinate investigation.

Fig. 2. Comparison of the model frequencies with observed infrared frequencies for (a) unsubstituted amide, (b) singly N-substituted amide in normal trans configuration, (c) cis amide, (d) doubly N-substituted amide. Shifts on deuteration are shown by an arrow with the shifted frequency indicated by a dashed line.

Figs. 4, 5, 6, 7, and 8 show the normal modes for five different amide models, and for the corresponding deuterated compounds, in the Amide I, Amide IIa and Amide IIb vibrations. A C-H bend is also shown for N-methyl formamide. The directions of the arrows show the direction of the motion of the atoms, when this is significant. The amplitude of the motion is indicated by the length of the arrow shaft, when it is large, and by the thick portion of the arrow shaft when it is small.

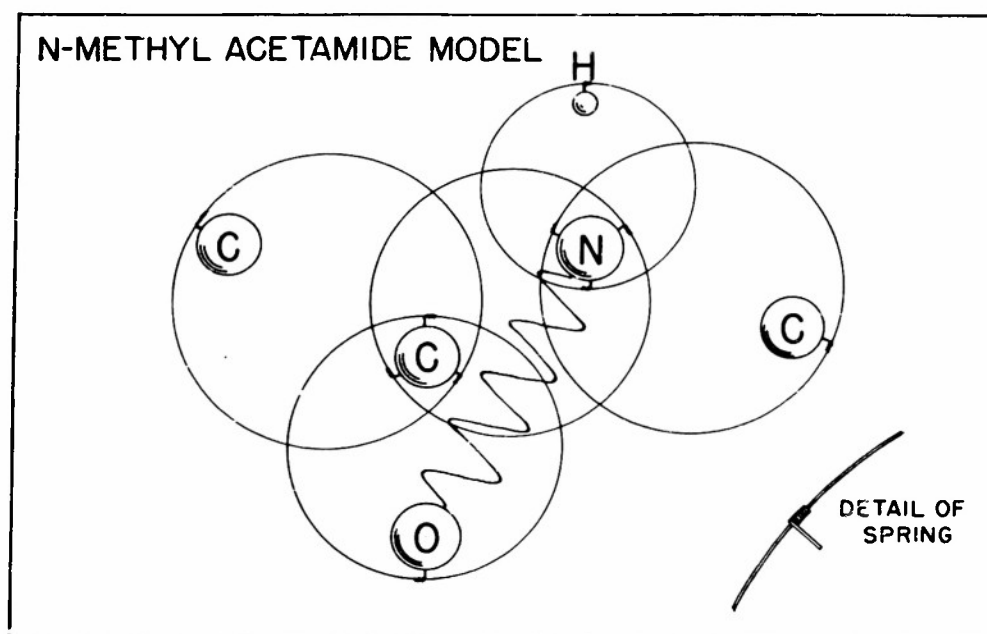
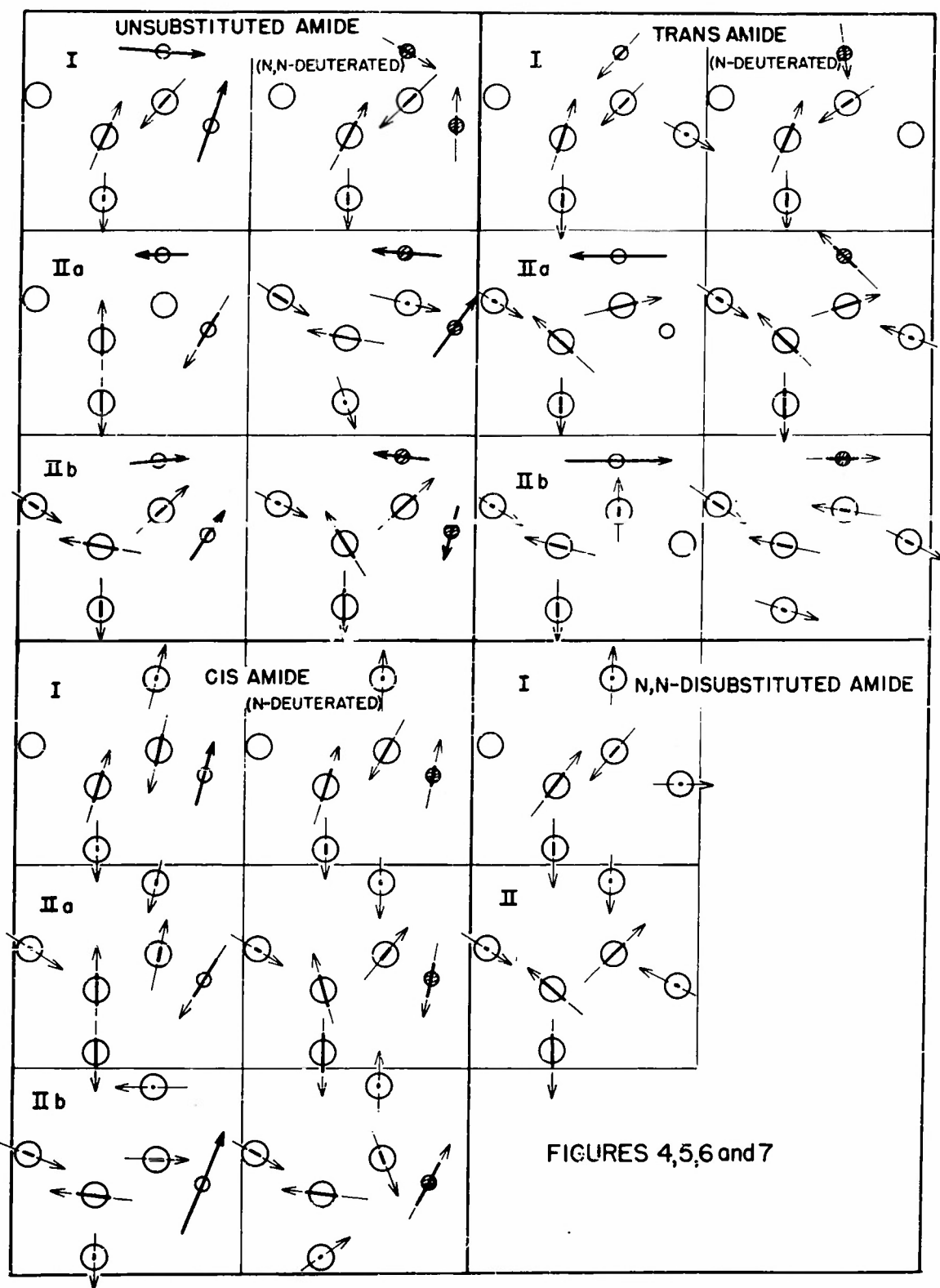
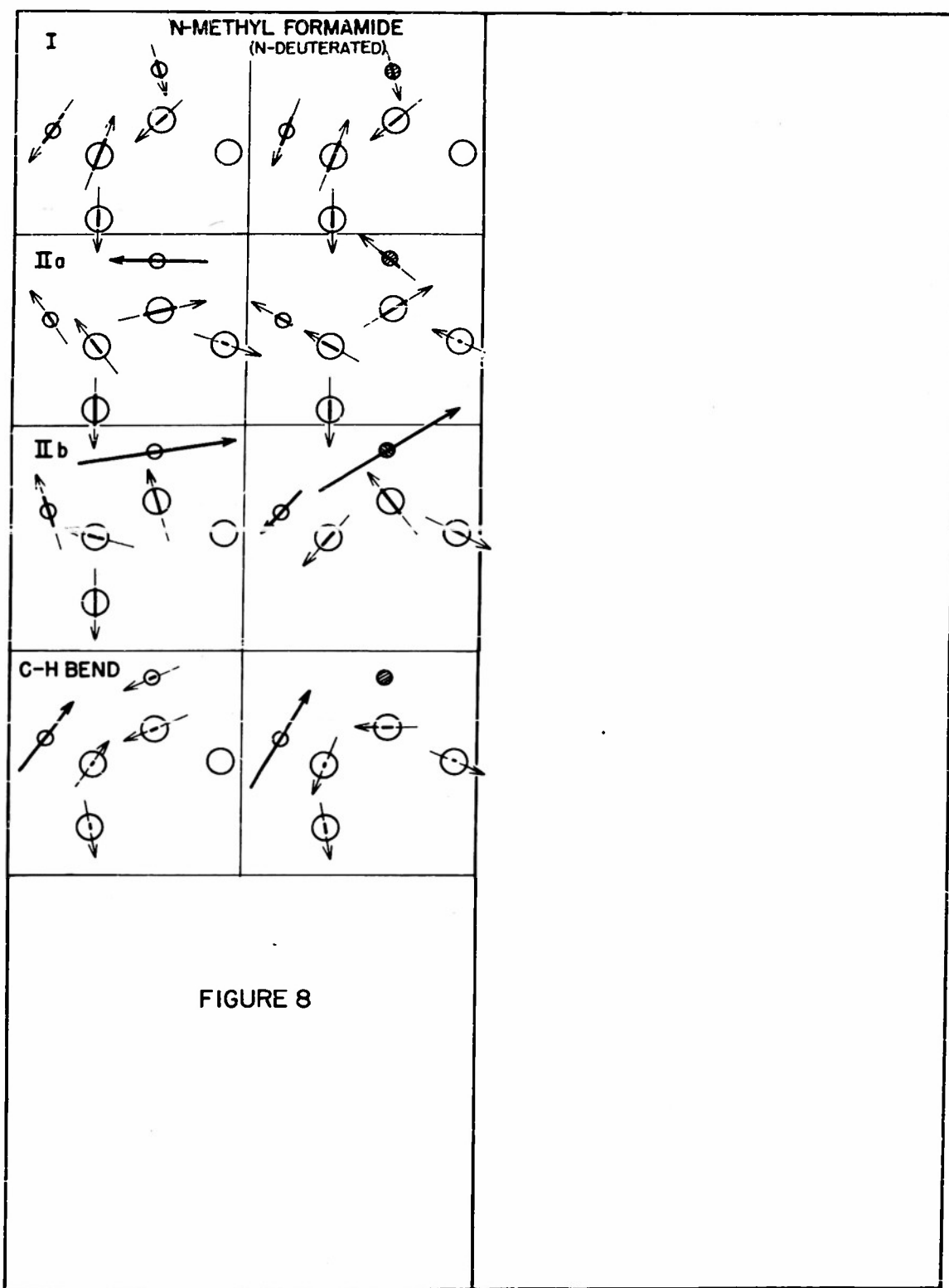


Figure 1

model	←		←			unsubstituted amide
observed						acetamide
model	←		←			N-substituted amide (trans)
observed	←		←			N-methyl acetamide
model	←					N-substituted amide (cis)
observed	?	←		?		diketopiperazine
model						N,N-disubstituted amide
observed				?		N,N-dimethyl formamide
1200 1400 1600 CM ⁻¹						

Figure 2





THE POLARIZED INFRARED SPECTRUM OF α DIGLYCINE.

PART I. GENERAL STUDY.

By A. D. E. Pullin

The polarized infrared spectra of single crystals of α diglycine and of NN' deuterated α diglycine have been obtained for two crystal planes, and the polarization behavior of a number of characteristic bands including those of the peptide group has been correlated qualitatively with the known bond directions.

Introduction

Three crystalline forms of diglycine have been described.¹ Recently detailed x-ray analyses of the structure of two of these, the α^2 and β^3 forms, have been made, thus offering the possibility of correlating polarized infrared spectra with previously known structural features and of comparing the infrared spectra of substances differing essentially only in the molecular packing and arrangement of hydrogen bonds.

The present paper describes the polarized infrared spectra of α diglycine and of α diglycine in which the hydrogen atoms attached to both nitrogen atoms have been replaced by deuterium, hereafter usually referred to as I and II respectively.

Crystal Structure

α diglycine belongs to the space group $P_{21/a}$. The molecules, existing as zwitterions, lie very nearly in planes parallel to (100) with the peptide group C=O and N-H bonds closely parallel to [010], the two-fold screw axis. Figs. 1, 2, and 3 show orthogonal projections of the unit cell on (100), (001) and (010).

Experimental

The α form of diglycine is invariably obtained on crystallization from aqueous solution. The main experimental difficulty lies in obtaining crystal sections thin enough to give useful polarized spectra, particularly in the $6\ \mu$ region. All the crystals used were grown from aqueous solution between either No. 1 microscope cover slips (for the $3\ \mu$ region) or silver chloride sheets, in both cases sandwiched between pyrex plates and lightly clamped together. The use of microscope cover slips gives, except for the thinnest crystals, well formed crystals of a uniform thickness, the crystals grown between silver chloride sheets being less uniform. The crystallization procedure used, depending mainly on slow evaporation, gave diamond shaped (001) plates. Occasionally (100) plates were found which were usually rectangular or nearly so, and always small. The probability of growing (100) crystals seemed to be greater for very slow crystallization. (001) and (100) crystals of II were obtained similarly from a solution prepared by evaporating diglycine with a large excess of D_2O five or more times. Both the deuteration and the crystallization were carried out in a dry box. It was found that the deuterium substituted crystals did not noticeably exchange in air (60% humidity) over a period of several weeks.

The initial identification of the crystal axes was kindly made for us by Dr. R. A. Pasternak from rotation and Weissenberg photographs, using a well formed (001) crystal. Thereafter all crystals used were examined optically. The (100) crystals, all grown between silver chloride sheets, clearly showed centred, or very nearly centred, acute bisectrix interference figures, as required by the optical data.¹ The optical properties of the (001) and (100) II crystals (optic sign, optic angle and the angle of rotation of the microscope

stage to cause the isogyres to leave the field) appeared identical to those of the corresponding crystals of I, showing that the same crystal form was being obtained. The interference colours given by the crystals between crossed polarizer and analyzer were used throughout as an aid in choosing crystals of the most suitable thickness. Approximate estimates of the thicknesses of the (001) crystals used are given in Figs. 4 and 7. These are based on values of 0.11_7 and 0.01_7^* for the birefringence of (001) and (100) crystal section respectively. The optical retardation of the (100) crystals was measured at numerous points on the crystals with a Berek compensator.⁴ All the (100) crystals sufficiently thin to give useful spectra were small necessitating the use of rather wide slits. The spectra for the (100) plane of II were obtained with a crystal 0.6-0.12 mm. The surroundings of the chosen crystal were masked out with a piece of 0.001" silver foil 4 mm. square in which a hole of the appropriate size had been scratched with a fine needle under a binocular microscope. The mask was stuck over a slightly smaller window cut in a strip of transparent adhesive tape and the strip and mask laid over the crystal adhering to the silver chloride sheet.** This was done with the silver chloride sheet fixed to the microscope stage, using low power magnification with the polarizer and gypsum red compensator inserted to increase the contrast.

Spectra were taken using a reflecting micro-illuminator⁵ in conjunction with a Beckman IR-2 spectrometer. The frequency of the 3288 cm^{-1} band was obtained during a series of measurements made with a vacuum grating spectrometer.⁶ A polarizer^{7,8} containing four sheets of silver chloride was used. The spectral slit widths used are shown on the spectra, Figs. 6 and 7.

*Calculated from the refractive indices of the (001) section and the optic angle, assuming α is \perp to (100).

**The silver chloride sheets were usually pulled apart after the crystallization was completed.

The Utilization of Infrared Polarization Data

For diglycine and similar crystals the unit cell vibrational modes will be approximately simple combinations of molecular modes. The symmetry of the crystal imposes the condition that these can only be in phase or 180° out of phase combinations of the motions of the four molecules comprising the unit cell. In general the different combinations will have different frequencies, so that the frequency of the combination of phases resulting in a unit cell mode polarized parallel to the symmetry axis [010] will be different from that of the combination polarized perpendicular to [010]. Usually the difference is small^{9, 10, 11} and is often not observed. Most of the bands for I and II listed in Table I show no significant differences in frequency for the two directions of polarization. For a doublet caused by this kind of interaction one component should be polarized strictly parallel to [010] and the other strictly perpendicular to [010]. Although it is possible to discuss the polarization spectra of I and II throughout in terms of unit cell modes it is equivalent and simpler to fix attention on a single molecule. It will be useful first to note some consequences of the crystal symmetry. Reference to Fig. 3 shows that polarization measurements on (010) crystals enable the directions of the components of the transition moments in this plane to be determined unambiguously, since in the (010) projection all corresponding directions of the molecules of the unit cell are either parallel or antiparallel. For planes containing the symmetry axis, such as (001) or (100) polarization measurements can only usefully be made with radiation polarized either parallel or perpendicular to it.* From such measurements two directions, equally inclined to

* By the phrase "radiation polarized parallel to" is meant radiation having the electric vector polarized parallel to.

the symmetry axes, are obtained for the directions of the projection of the transition moment in the plane being examined, in turn leading to two choices, with respect to the molecule, for the direction of the projection of the transition moment in this plane. The choice of the most suitable pairs of planes for polarization measurements will generally be determined by the symmetry of the crystal.^{12, 13} In the case of a diglycine, the most suitable would be (100) and (010). However no (010) crystals were found using the crystallization procedure described, although a large number of crystals were examined by means of their conoscopic interference figures. If polarization data for (001) and (100) crystals are combined, in general four choices are obtained for the direction of the transition moment of each absorption band. Because of this ambiguity, only qualitative use has been made of the present polarization data for these (001) and (100) crystals, except in the case of ν (N-H) (Part II) for which the four possible directions lie within a small solid angle, and for a number of bands for which the polarization data require the transition moment to lie at an angle of 30° or more with (100). These are noted in Tables I and II. For the reasons given all 'out of plane' bands need not and probably have not been detected. It should also be noted that from the polarization data for the (100) and (001) planes it is not possible to be certain that the transition moment for a particular band lies in (100), i.e. approximately in the molecular plane. It will be convenient to define dichroic ratios for each plane with the absorption for radiation parallel to [010] in the numerator and for radiation polarized in the plane in question and perpendicular to [010] in the denominator.

$$\text{i.e., Dichroic ratio for (100) = D.r.}_{(100)} = \frac{\int \epsilon_{[010]} d\nu}{\int \epsilon_{[001]} d\nu} *$$

*Usually the ratio of band maxima instead of band areas is measured. This procedure has been followed here.

$$\text{Dichroic ratio for (001)} = D.r_{(001)} = \frac{\int \epsilon_{[010]} d\nu}{\int \epsilon_{[100]} d\nu}$$

where $\epsilon_{[010]}$ etc. are the extinction coefficients for radiation polarized along these axes and incident normally on the plane in question, and the integration is taken over the entire absorption band. It may easily be shown that for a band whose transition moment lies in the (100), or approximate molecular plane:

$$D.r_{(100)}/D.r_{(001)} = \cos^2 \beta = 0.33^{14}$$

where β is the angle ($124^\circ 35'$) between (100) and (001). However the same value of the quotient $D.r_{(100)}/D.r_{(001)}$ should also be found for a band whose transition moment is in a plane which contains [010] and lies between [100] and [001] making an angle of θ with the latter, such that

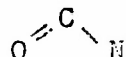
$$\frac{\cos^2 (\beta - \theta)}{\cos^2 \theta} = \cos^2 \beta = 0.33 \quad \text{This gives } \theta \approx 55^\circ$$

Discussion of the Spectra

The spectra obtained for (001) and (100) crystals of I and II are given in Figs. 6 and 7 and the corresponding frequencies are listed in Table I. Polarized infra-red spectra of I for the region $1400\text{--}1700\text{ cm}^{-1}$ and $2900\text{--}3400\text{ cm}^{-1}$ have been given by Abbott and Ambrose.¹⁵ Their spectra are essentially the same as ours for the (001) crystals. Spectra of mulls of polyglycine, glycine, diglycine, etc., through to penta or hexaglycine have been published by Thompson, Nicholson and Short¹⁶ and by Blout and Linsley.¹⁷ Lenormant¹⁸ and Kellner¹⁹ have also published spectra of diglycine. These and numerous other related studies have made the attribution of a number of the bands of the diglycine spectra reasonably certain. Such attributions are noted in

Table I and are not further discussed except where the present polarization data have given further information.

From the point of view of the relation of the spectra of I and II to those of the natural polypeptides the most interesting spectral region is at $6\ \mu$. Recently there has been discussion concerning the attribution of the bands at ~ 1650 and ~ 1550 found in all peptides,^{20,21,22} hereafter called Amide I and Amide II respectively. The most recent proposal is that of Price and Fraser²² who discussed the normal modes of the peptide group in terms of the bent tri-atomic group



Using this approximation they related the antisymmetric stretching vibration of this group to Amide I and the pseudo-symmetric stretching mode to the band at $\sim 1490\ \text{cm}^{-1}$ observed in deuterium substituted peptides.^{21,23,24} In the absence of deuterium substitution Price and Fraser consider the pseudo-symmetric mode to interact with the N-H bending mode to give Amide II at $\sim 1550\ \text{cm}^{-1}$ and a vibration at a considerably lower frequency, probably at $1270\ \text{cm}^{-1}$. On the basis of this scheme for the normal modes they suggested that the transition moment of Amide I lies in the OCN plane somewhere between the OC and CN directions. Several experiments to determine this direction have been made.^{25,26}

α diglycine is a favorable substance for this purpose, because of the near coincidence of the directions of the C=O bonds and $[010]$, or symmetry axis. Our (001) spectra of I, and those given by Abbott and Ambrose¹⁵ indicate that the component of the transition moment of Amide I in a direction perpendicular to the C=O bond must be considerable, for the absorption in this direction in the (001) spectra should be reduced by a factor of ~ 3 because of the inclination of the plane of the peptide group with (001).¹⁴ This indication is reinforced by the (001) spectra of II where the difficulty of the overlapping of Amide I with a band at $1635\ \text{cm}^{-1}$ is avoided. These spectra suggest that the

transition moment might be sufficiently inclined from [010] as to lie along the C-N direction.

Unfortunately all the (100) crystals were too thick for this spectral region but their spectra are consistent with the transition moment of Amide I being about equally inclined to [010] and [001], that is, nearly along (or nearly perpendicular to) the C-N direction. Because of the smallness of the (100) crystals a compromise was made between masking out all holes and in so doing considerably reducing the available area, and allowing a few holes. These amounted to only a few percent of the total area. The (001) crystals were masked to avoid holes altogether but a quantitative interpretation of the polarization data has not been made for the following reasons:

1. Part of the observed absorption is actually caused by selective reflection.²⁷
2. Use of too wide slit widths.
3. See reference 13.
4. Non uniformity of sample thickness.

Apart from the uncertainties of measurement there is the question of how much the transition moment may vary from compound to compound.²⁶ It is conceivable that diglycine may not be very typical of peptides in general in this respect because of the possibility of interaction of Amide I with the vibration at 1635 cm^{-1} (an NH_3^+ mode) and the carboxylate ion stretching mode at $\sim 1612\text{ cm}^{-1}$. The interaction with the 1635 cm^{-1} vibration may be important as the frequency of Amide I is $\sim 12\text{ cm}^{-1}$ lower in II. This is a greater change than is usually found.

The spectral region below Amide I is complicated by the uncertainty regarding the location of the NH_3^+ bands and the degree of splitting in diglycine of the modes degenerate under C_{3v} symmetry. An NH_3^+ group of C_{3v} symmetry would be expected to give^{28,29,30} two bands in the region $1500\text{--}1650\text{ cm}^{-1}$.

corresponding to the symmetric and antisymmetric bending modes, a lower frequency band probably in the region $900\text{--}1300\text{ cm}^{-1}$ corresponding to the degenerate rocking modes, and a further, probably lower frequency band, corresponding to the torsional mode. The band at 1635 cm^{-1} in I and absent in II can be assigned to one of the NH_3^+ bending modes. It seems necessary to assume that interaction between Amide I at 1582 cm^{-1} and the carboxylate ion vibration at 1612 cm^{-1} has raised the latter $\sim 7\text{ cm}^{-1}$ as in II it is at 1595 cm^{-1} . In II a band is found at $\sim 1490\text{ cm}^{-1}$, polarized perpendicular to $[010]$, while in I there is a band at 1485 cm^{-1} polarized parallel to $[010]$. This clearly is an entirely different mode. The occurrence of the 1490 cm^{-1} band in II is in agreement with other observations on deuterated peptides,^{21,23,24} and may, following Price and Fraser,²² be assigned to the pseudo symmetric stretching mode of the $\text{O}=\text{C}-\text{N}$ group.

The 1485 cm^{-1} band in I, along with bands at 1392 cm^{-1} and 1340 cm^{-1} , absent in the spectra of II may be plausibly assigned to bending and rocking modes of the NH_3^+ group. The assignment of bands at 1447 and 1411 cm^{-1} to the CH_2 scissors vibration and the lower frequency carboxylate ion vibration respectively follow from their well known general location and from their presence in the spectra of both I and II. Similarly the band at 1316 cm^{-1} present in the spectra of I and II and polarized perpendicular to $[010]$ can probably be assigned to the CH_2 wagging vibration.

The polarization of some of the bands in this region is unexpected. Thus both carboxylate ion stretching modes would be expected to show little polarization in the (100) spectra whereas that at 1612 cm^{-1} is well polarized parallel to $[010]$. This may be connected with the strong intermolecular hydrogen bonding between the carboxylate ion group and the NH_3^+ groups. The polarization of the CH_2 scissors mode at 1467 cm^{-1} is not as complete as would be expected. Blout and Linsley¹⁷ remarked on the wide variations of intensity

shown by this band in the series of glycyl peptides they studied, and the two effects may be connected.

From the relative absorption maxima of the stretching vibrations of NH_3^+ and ND_3^+ , peptide NH and ND, there appears to be $\sim 15\%$ residual light hydrogen in the crystal of II.* From this, assuming random substitution of H by D in the NH_3^+ groups, it follows that II should contain $\sim 61\%$ of ND_3^+ groups, $\sim 11\%$ each of the three kinds of ND_2^+H groups, $\sim 2\%$ each of the three kinds of NDH_2^+ groups, and a negligible amount of NH_3^+ . Thus the effect of the incompleteness of the deuteration on the spectra of II should be to add a number of weak satellite bands while still showing the features of the ND_3^+ spectrum. Comparison of the spectra of I and II shows that while the spectra of I contains intense bands polarized parallel to [010] at 1635, 1485 and 1392 cm^{-1} and a weak out of plane band at 1340 cm^{-1} , which can all be plausibly assigned to NH_3^+ bending and rocking modes, it is not possible to find analogues of these of comparable intensity in the spectra of II, though there are weak bands polarized parallel to [010] at 1049 and 997 cm^{-1} . The most likely explanation seems to be that considerable interaction takes place between the deuterium bending vibrations and the skeletal vibrations. Such interaction would also account for the absence in the spectra of II of a strong band in the region 1050-1150 cm^{-1} polarized strictly perpendicular to [010] corresponding to the peptide group ND bending vibration.

The present polarization and intensity data suggest that the weak bands at ~ 1239 and 1249 cm^{-1} in II may correspond to weak bands in I at 1240 and 1258 cm^{-1} respectively. It is further possible that in each case the pair of

*This is probably an overestimate: the absorption from vibrations involving mainly deuterium motion should be intrinsically less strong than that from the corresponding hydrogenic vibrations.

bands may represent a single molecular mode split by interaction with the other molecules of the unit cell. This origin would require the components of each pair to be completely and oppositely polarized, that is parallel and perpendicular to $[010]$. The polarization of these bands is not very clear but the observations do not seem to be inconsistent with this requirement. The polarization of the molecular mode would then be given by the ratio of intensity of the lower frequency parallel component to the higher frequency perpendicular component. For the (001) spectra of I and II the lower frequency component is somewhat more intense, while in the (100) spectra of I and II the higher frequency component is several times more intense. The intensities of the corresponding bands of I and II appear to be similar. These bands may arise from a vibration of the peptide group part of the molecule since there is believed to be a frequency characteristic of the peptide group in the region 1200-1300 cm^{-1} ,^{22,24} but there is some evidence²⁴ that this characteristic band disappears from this region on deuteration.

The polarization data also suggest that bands at 1007 and 1022 cm^{-1} in I and II respectively may have a similar origin. If this is so, the small shift on deuteration would be consistent with the suggestion of Blout and Linsley¹⁷ that a band observed by them at 1000-1025 cm^{-1} in a number of glycine peptides has a common origin in a skeletal vibration of a portion of unsubstituted peptide chain.

The polarization of the band at 710 cm^{-1} in I is striking in that its transition moment appears to be almost exactly perpendicular to (100), Fig. 4. The polarization of the nearest band in the spectra of II, at 704 cm^{-1} is quite different, so that it is plausible to attribute the 710 cm^{-1} band in I to the out of plane vibration of the peptide NH group which would be expected to show exactly this polarization. This conclusion is in agreement with other observations on bands in this region^{17,24} in related compounds.

We cannot find any satisfactory explanation of the moderately strong band at 1361 cm^{-1} in II, except that it may arise from a superposition of NH bending vibrations from the residual hydrogen in NHD_2^+ and NH_2^+D groups in II.

Of the bands not attributable to fundamental vibrations that at 1688 cm^{-1} is of particular interest because of the occurrence of a band of nearly the same frequency in the spectrum of silk fibroin, where its interpretation is of importance in connection with structural investigations of that substance. A band at this frequency is not generally observed in polypeptide spectra, but appears to be characteristic of the glycine residue. Blout and Linsley¹⁷ have observed a band at $\sim 1688\text{ cm}^{-1}$ in several tripeptides containing a single glycine residue in any of the three possible positions. They also found it in various polymers of glycine and their published spectra do not seem to us to support an observation by Elliott³¹ that the intensity diminishes with increasing molecular weight.

From the evidence mentioned the 1688 cm^{-1} band seems to be associated with an amide group in which a methylene group is attached to either the peptide nitrogen or carbon. The possibility that it is a fundamental frequency of this group seems to be excluded and the combinations and overtones to which it can be attributed are limited by several facts. Neither the frequency nor the intensity of 1688 cm^{-1} are greatly altered by deuteration which excludes strong participation of Amide II and N-H bending modes. The parallel polarization in silk fibroin shows that the change in dipole moment in the vibration is at least nearly parallel to the molecular chains which makes it unlikely that any "out of plane" vibration is involved. No suitable fundamental at 844 cm^{-1} seems probable and although an in plane amide bend^{32,33} probably lies near 563 cm^{-1} it is not obvious why the third overtone should be associated exclusively with a glycine residue.

For the reasons given it seems very plausible to assign 1688 cm^{-1} to a combination of the 1316 cm^{-1} CH_2 wag with some vibration in the neighborhood of 372 cm^{-1} , which is probably to be described as a N-C-C bend.

Acknowledgement

We wish to acknowledge our indebtedness to Dr. E. W. Hughes who has given us prior to publication the information regarding the structure of a diglycine, without which the work described in this section would not have been possible.

Table I. (Continued)

cm. ⁻¹	^α Diglycine (001) //:⊥ (100) //:⊥	Assignment	cm. ⁻¹	NH ₄ ⁺ Deuterated α Diglycine (001) //:⊥ (100) //:⊥	I Assignment
1584	(001) //:⊥ (100) //:⊥	Amide II			
1550	-	W			
1505	-	VW	1545	<	VW
1485	>>	S	1490	>	MS
1447	>	MS	1446	>	MS
1411	>	S	1412	>	S
1392	>>>	S	1361	>	MS
1340	≈	MS ^b			
1316	<<	MS	1315	<	MS
1258	<<	W	~1249	<	W
1240	>>	W	~1239	(>)	W
1164	<	MS ^b	1172	>	S
1140	>	MS	1155	<	?
1103	>	W			
1050	<	VW			
1007	>	MS	1049	>	W
			1022	>	W
			997	>	W

Table I. (Continued)

cm. ⁻¹	α Diglycine		Assignment	cm. ⁻¹	NNL-Deuterated α Diglycine		
	(001) //:⊥	(100) //:⊥			(001) //:⊥	(100) //:⊥	I Assignment
968	<	≪	MS	935	<	<	W
922	<	≈	sb				
903	<	>	MS ^b				
735 ^d	>	>	W	845	<	-	WW
725-715 ^d	-	-	WW	822	<	-	WW
710 ^{d,e}	≪≪	No absorption	S ^b	787	<	≈	MS ^b
			Out of plane NH bend	704	>	>	S

- a. The inequalities indicate the relative intensities parallel and perpendicular to [010].
- b. The transition moment makes an angle of 30° or more with (100).
- c. This band was clearly resolved on one spectrum obtained.
- d. See Fig. 4. There may be two bands, at 715 and 725 cm.⁻¹.
- e. The band at 710 cm.⁻¹ strictly \perp to (100).

References for Section 2, Part I

1. L. Bernal, Z. Krist., 78, 363 (1931).
2. E. W. Hughes, to be published.
3. E. W. Hughes and W. J. Moore, J. Am. Chem. Soc. 71, 2618 (1949).
4. Wahlstrom, Optical Crystallography, John Wiley and Sons, New York and Chapman and Hall, London. See p. 107, 1st Edition (1943), p. 132, 2nd Edition (1951).
5. R. M. Badger and R. Newman, Rev. Sci. Instruments, 22, 935 (1951).
6. R. M. Badger, L. R. Zumwalt and P. A. Giguere, *ibid.*, 19, 861 (1948).
7. R. Newman and R. S. Halford, Rev. Sci. Instruments, 19, 270 (1948).
8. N. Wright, J. Opt. Soc. Am., 38, 69 (1948).
9. D. F. Hornig, Far. Soc. Disc., 9, 115 (1950).
10. G. C. Pimentel and A. L. McLellan, J. Chem. Phys., 20, 270 (1952).
11. R. S. Stein, G. B. B. M. Sutherland, J. Chem. Phys. 21, 371 (1953).
12. R. Newman and R. S. Halford, J. Chem. Phys. 18, 1277 (1950).
13. R. Newman and R. M. Badger, J. Chem. Phys., 19, 1148 (1951).
14. The exactness of this relation may be impaired under conditions of the kind discussed in references 12, and 13. Data on this point are not available.
15. N. B. Abbott and E. J. Ambrose, Proc. Roy. Soc. (London) A 219, 17 (1953).
16. H. Thompson, D. Nicholson and L. Short, Disc. Far. Soc. 9, 232 (1950).
17. E. R. Blout and S. G. Linsley, J. Am. Chem. Soc. 74, 1946 (1952).
18. H. Lenormant, C. r. Acad. Sci. (Paris) 221, 565.
19. L. Kellner, Proc. Roy. Soc. (London) A 177, 447 (1941).
20. G. B. B. M. Sutherland, Far. Soc. Disc. 9, 280 (1950).

21. H. Lenormant, *Annal. Chim.* 5, 459 (1950).
22. R. D. B. Fraser, W. C. Price, *Nature* 170, 490 (1952).
23. H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangel, Infrared Determination of Organic Structures, D. Van Nostrand Company, Inc., New York (1949).
24. H. B. Kessler, Ph.D. Thesis, University of Michigan, 1953.
25. C. H. Bamford, L. Brown, A. Elliott, W. E. Hanby and I. F. Trotter, *Proc. Roy. Soc. (London) B* 141, 49 (1953).
26. A. E. Elliott, *Nature* 172, 359 (1953).
27. The reflection spectra of diglycine and a discussion of the effect of reflection on absorption is discussed in Section 6 of this report.
28. R. Ananthakrishnan, *Proc. Ind. Acad. Sci. A* 5, 76, 87, 200 (1937).
29. J. T. E. Edsall and H. Scheinberg, *J. Chem. Phys.* 8, 520 (1940).
30. H. Lenormant, *J. Chim. phys.*, 43, 326 (1946).
31. A. Elliott, *Proc. Roy. Soc. (London) A* 221, 104 (1954).
32. R. Newman and R. M. Badger, *J. Am. Chem. Soc.*, 74, 3545 (1952).
33. R. D. Waldron, Ph.D. Thesis, California Institute of Technology, 1952.

THE POLARIZED INFRARED SPECTRUM OF α DIGLYCINE
PART II TRANSITION MOMENT DIRECTION OF THE N-H VALENCE VIBRATION

By A. D. E. Pullin

INTRODUCTION

A knowledge of the direction of the transition moment associated with the peptide N-H stretching vibration is of considerable importance for the infrared studies of proteins. The only previous determination is that of Newman and Badger¹ for acetylglycine for which they found that the angle between the transition moment and the N-CH₂ bond was $100^\circ \pm 10^\circ$. The high dichroic ratio of the N-H stretching vibration observed at low dispersion in (001) and (100) crystals of α diglycine² suggested that the transition moment direction must be nearly parallel to [010]. In order to define the direction more accurately the efficiency of the polarizer was increased by using six sheets of silver chloride and all measurements were made at high dispersion using a grating spectrometer.³

Basis of Method

Essentially the method was based on absorption measurements on thin (001) and (100) crystals for radiation polarized parallel to [010] and on thicker (001) and (100) crystals for radiation polarized perpendicular to [010], followed by a determination of the ratio of the thicknesses of the thin and thick (001) crystals and the thin and thick (100) crystals. A number of corrections have to be considered, of which the most serious is that arising from strong selective reflection occurring with the absorption. For assumed values of the extinction coefficients and refractive index it is possible to calculate the errors introduced if absorption measurements are made without correcting for reflection.⁴

To do so it is necessary to know the thickness of the absorber, and its true extinction coefficient and refractive index at the maximum of the absorption band. The latter two constants are not known for diglycine so the procedure was adopted of taking reasonable values for these constants based on known values for absorption bands of other substances giving rise to reflection bands of the same intensity as the of ν_{NH} in a diglycine.⁴ Using these constants it was found that extinction values measured for crystals of a diglycine for radiation polarized parallel to [010] (and thereby very nearly parallel to the N-H bond) could be too large by a factor of ~ 1.6 if no corrections were made for reflection.

Experimental

(a) (001) Crystals

The experimental results are summarized in Table I. The ratio of the thicknesses of the (001) crystals was obtained from their relative optical retardations using a Sénarmont compensator⁵ for the thin crystal and a Berek compensator⁶ for the thick crystal. No absorption was detected for radiation polarized perpendicular to [010] with the thick crystal. It was not possible to use a thicker crystal because the absorption from the wings of longer wavelength bands was already troublesome. It seemed very unlikely that as much as 3% absorption could remain undetected. Support for this limit was obtained by turning the polarizer slightly from the position for perpendicular polarization. This allowed a small component of parallel polarized radiation to pass which being essentially all absorbed, produced an absorption band of known intensity. It was found that the extra absorption so produced could be detected when the polarizer was turned on either side of the perpendicular position so as to give $\sim 2\%$ absorption. For the calculation of dichroic ratio it was assumed

that the absorption of radiation perpendicular to [010] for the third crystal could not be greater than 3%.

(b) (100) Crystals

For (100) crystals, reflection difficulties were circumvented by making the measurements on the residual NH in crystals of deuterated diglycine in which the γ NH extinction coefficient was insufficient to cause appreciable errors from reflection. As it was not known if the percentage of residual NH in both crystals was the same, measurements based on optical retardation could not be used to determine their relative thickness. Instead the NH absorption band for radiation polarized parallel to [010] was obtained for both crystals at high linear dispersion, wavelength markings being made automatically at $\sim 3 \text{ cm}^{-1}$ intervals. Ratios of the extinctions of the two bands were then obtained at regular intervals on the sides of the bands over a range of a factor of 3 in extinction values. The mean of these gave a ratio of effective thicknesses of 4.5 with a standard deviation of 0.3 units.

The thin crystal gave a high (90.5%) maximum absorption for radiation polarized parallel to [010]. High absorption values are especially sensitive to errors from stray light and other errors in locating zero transmission. That this was being done correctly in this case was shown by the coincidence of the zero transmission position and the maximum of the absorption band of the thick crystal for radiation polarized parallel to [010].

The absorption of the thick crystal (8%) for radiation polarized perpendicular to [010] was an averaged value of a number of determinations at or near the position for minimum absorption. From the measurements on the (001) crystals, the maximum amount of the wrong component passed by the polarizer could not be greater than 3%, so that the absorption of the thick (100) crystal for radiation polarized perpendicular to [010] was taken as 5-8%.

Discussion of Results

(a) Experimental error.

Errors of measurement due to the convergence of the radiation on the crystal, finite slit width and crystal imperfections would all decrease the observed dichroic ratio. Some experiments in which the angular aperture of the beam falling on the crystal was reduced indicated that errors arising from convergence were small. The slit widths used were small compared with the half band widths. The (100) crystal used was well formed, gave sharp extinction and appeared free from mosaic structure when examined with the optical microscope. It is believed that the absorption of radiation polarized perpendicular to [010] is not due to mosaic structure.

(b) Interpretation.

As discussed in part I, the dichroic ratios of an absorption band for the (001) and (100) planes do not define a unique direction for the corresponding transition moment, but give four choices for this direction. In the present case, the ranges of possible values for the dichroic ratios for the two planes define solid angles within which the transition moment must lie. Fig. 5 shows a projection of the peptide group of α -diglycine on a plane perpendicular to the plane of the nitrogen and two adjacent carbon atoms, and perpendicular to the bisector of the CNC angle. The shaded areas indicate the solid angles within which the transition moment must lie and are delineated by the projection on the plane of a point 1 A from the nitrogen atom along the transition moment, as defined by the ranges of the dichroic ratios for (001) and (100). That is, the shaded areas represent two possible ranges of positions of the projection

of an "effective H" of \angle NH.

The choice for which the transition moment may lie almost in the C(3), N(6), CH₂(2) plane, making an angle of $\sim 110^\circ$ with CH₂-N seems more likely. The deviations of the four heavy atoms O(7)-CH₂(2) from one common plane are probably not significant and Fig. 5 would be little affected by choosing as reference plane instead the least mean squares plane through O(7)-CH₂(2) or the plane through O(7), C(3), N(6). (In this case the out of plane angles are all reduced by $\sim 1^\circ$.) The five heavy atoms CH₂(1)-CH₂(2) deviate significantly from a common plane but the deviations from a common plane for CH₂(1), C(3), O(7), N(6) are probably within the experimental errors. If the transition moment direction is referred to this plane, the out of plane angles are increased by 2° - 3° over those shown in Fig. 5. There seems little reason to expect the transition moment to deviate from the C(3), N(6), CH₂(2) plane. The two atoms most likely to affect both the position of the hydrogen atom and the direction of the transition moment with respect to the N-H bond are O(3) and the O(3) of the neighboring molecule to which N(6) is hydrogen bonded, but the former lies in a common plane with C(3), N(6), CH₂(2) and the latter only deviates by $\sim 3^\circ$ from this plane.

This choice is in agreement with the observations of Newman and Badger on acetylglycine.¹ On either alternative the results are not consistent with the transition moment being directed towards the oxygen to which the nitrogen is hydrogen bonded.

It is possible that the transition moment deviation may differ significantly from the N-H bond direction due to increasing contribution of the ionic structure with increasing N-H distance during vibration.

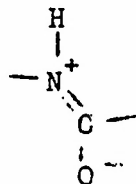


Table I

	Crystal			
	Thin (001)	Thick (001)	Thin (100)	Thick (100)
Absorption \parallel [010]	82% (apparent)	-	90.5%	-
Extinction " "	0.465 (corrected)	-	1.022	-
Absorption \perp "	-	∇ 3%	-	5-8%
Extinction " "	-	∇ 0.013 ₂	-	0.022 ₃ -0.036 ₂
Ratio of thicknesses	7		4.5	
Dichroic Ratio	\nwarrow 250:1		205-127:1	
Corresponding angle with [010]	0-3 1/2°		4°-5°	

References for Section 3, Part II

1. R. Newman and R. M. Badger, J. Chem. Phys. 19, 1147 (1951).
2. See Part I, Figures 1 and 2.
3. Reference 6, Part I of Section 3.
4. Reference 27, Part I of Section 3.
5. Hartshorne and Stuart, Crystals and the Polarizing Microscope, 2nd Edition, Edward Arnold and Co., London, 1950. See page 453.
6. Wahlstrom, Optical Crystallography, John Wiley and Sons, New York, and Chapman and Hall, Ltd., London. See p. 107, 1st edition (1943); p. 132, 2nd. edition (1951).

Captions for Figures of Section 3, Parts I and II

- Fig. 1. Orthogonal projection of the unit cell of a α diglycine onto (100). Only the molecular layer at $x \approx 1/4$ is shown except for some atoms of other layers connected by hydrogen bonding. These are shown in broken lines. The intersection of $x \approx 1/4$ and the unit cell faces bound the figure. We are indebted to Dr. E. W. Hughes for use of data from which Figures 1, 2 and 3 were drawn.
- Fig. 2. Orthogonal projection of part of the unit cell of a diglycine on (001). Only the molecular layer at $x \approx 1/4$ is shown, except for atoms of other layers connected by hydrogen bonding. These are shown in broken lines.
- Fig. 3. Projection of the unit cell of α diglycine onto (010). The molecules shown in full lines are above those shown in broken lines.
- Fig. 4. $\text{NH}_3^+\text{CH}_2\text{CONHCH}_2\text{CO}_2^-$ 14μ region. Extinction curves for (001) and (100) crystals for radiation \parallel and \perp to [010].
- Fig. 5. (a) Diagram of a single molecule in a diglycine. The NH_3^+ group is below the plane of the rest of the molecule. (b) Projection of part of the molecule down onto a plane perpendicular to the bisector of the angle $\text{CH}_2(2)$. $\text{N}(6)$ $\text{C}(3)$. The shaded areas represent the range of possible directions for the transition moment of νNH with respect to this plane, and are on the same side of this plane as the NH_3^+ group.
- Fig. 6. Spectra of α $\text{NH}_3^+\text{CH}_2\text{CONHCH}_2\text{CO}_2^-$. (100) and (001) crystals. Radiation \parallel and \perp to [010]. (100) spectra obtained with a crystal $\sim 7 \mu$ thick. (001) spectra with a crystal $\sim 2 \mu$ thick except for curves (a) $\sim 6 \mu$ thickness, and (b) $< 1 \mu$ thickness.

Fig. 7. Spectra of α $\text{DH}_3^+\text{CH}_2\text{CONDCH}_2\text{CO}_2^-$. (100) and (001) crystals. Radiation \parallel and \perp to $[010]$. (100) spectra obtained with a crystal $2.6 - 4.2$ μ thick. (001) spectra obtained with a crystal approximately $1 - 1.5$ μ thick, except for curves labelled (a) obtained with a considerably thicker crystal. Bands from $10 - 15$ μ in the (001) spectra checked against or calculated from the spectra of thicker crystals.

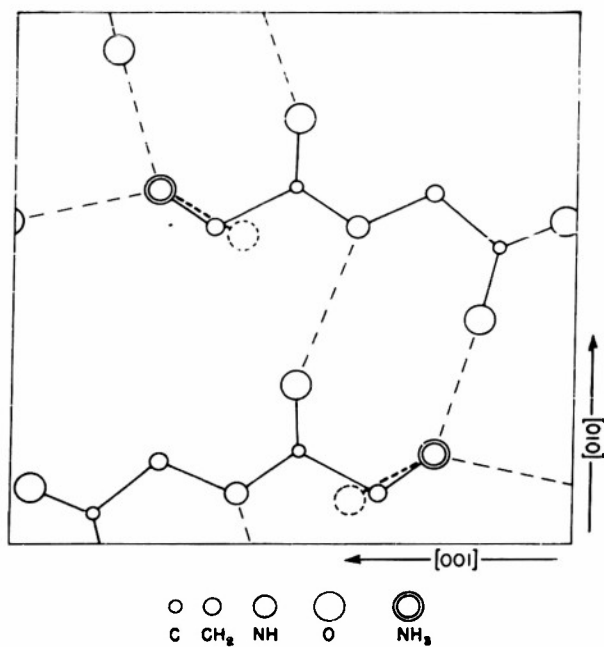


Figure 1

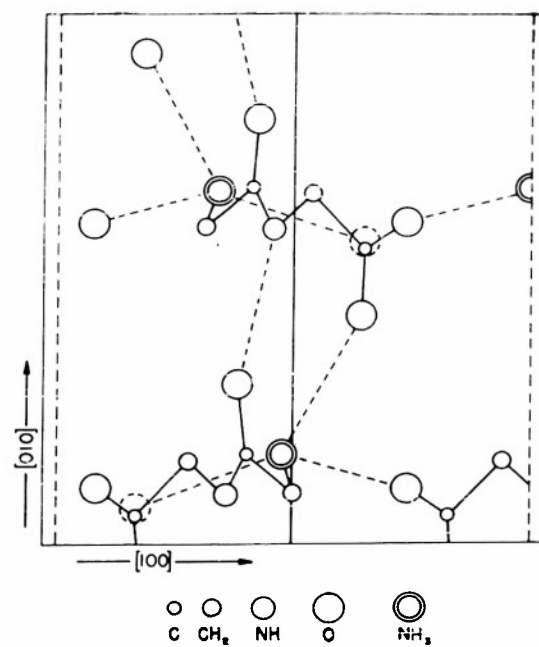


Figure 2

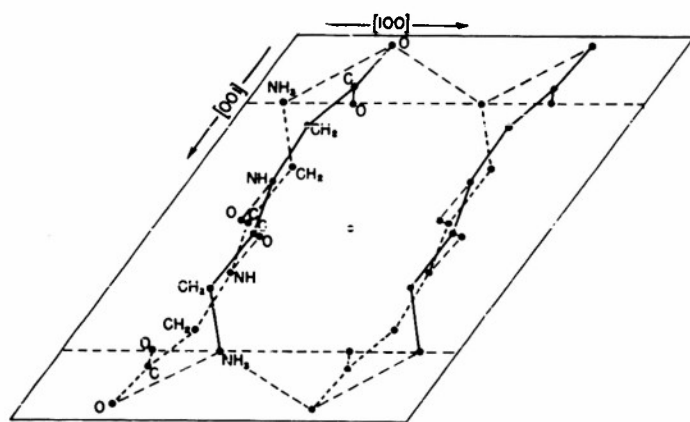


Figure 3

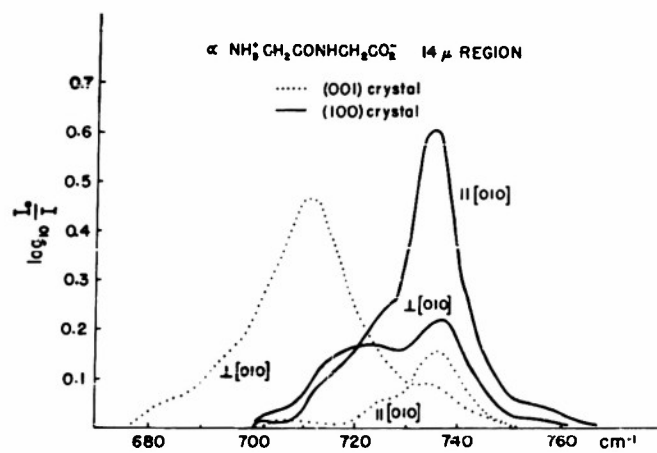


Figure 4

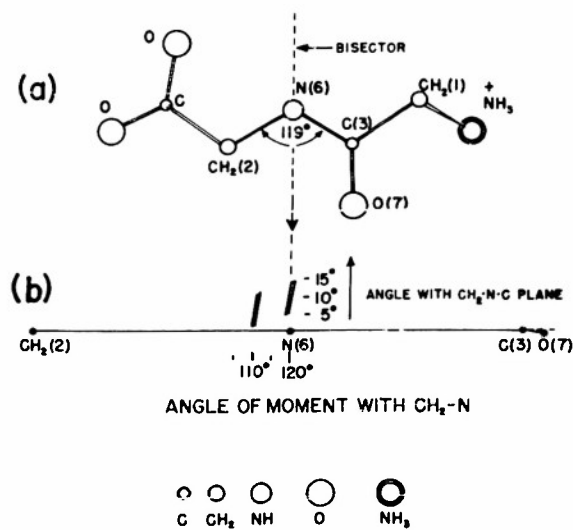


Figure 5

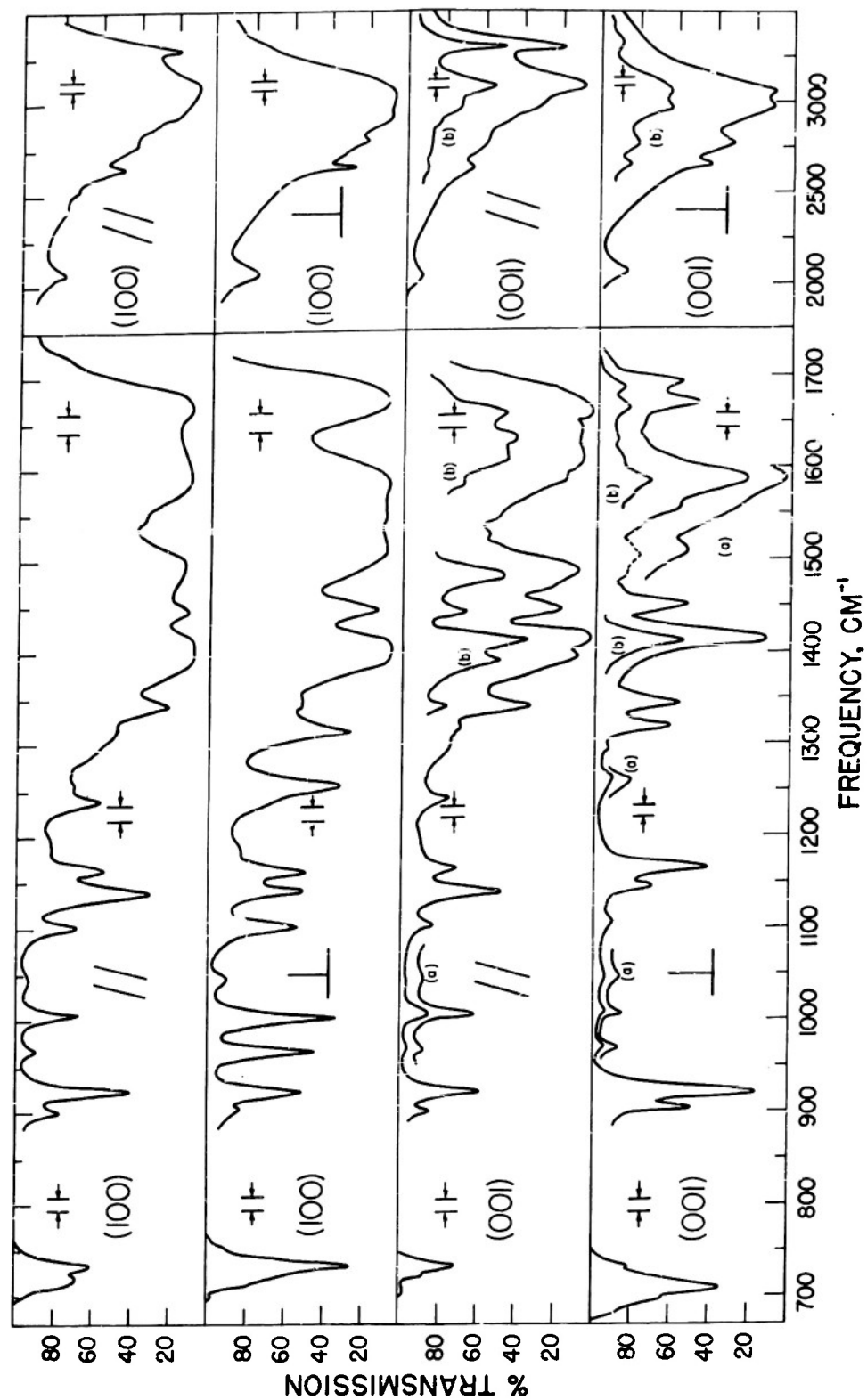


Figure 6

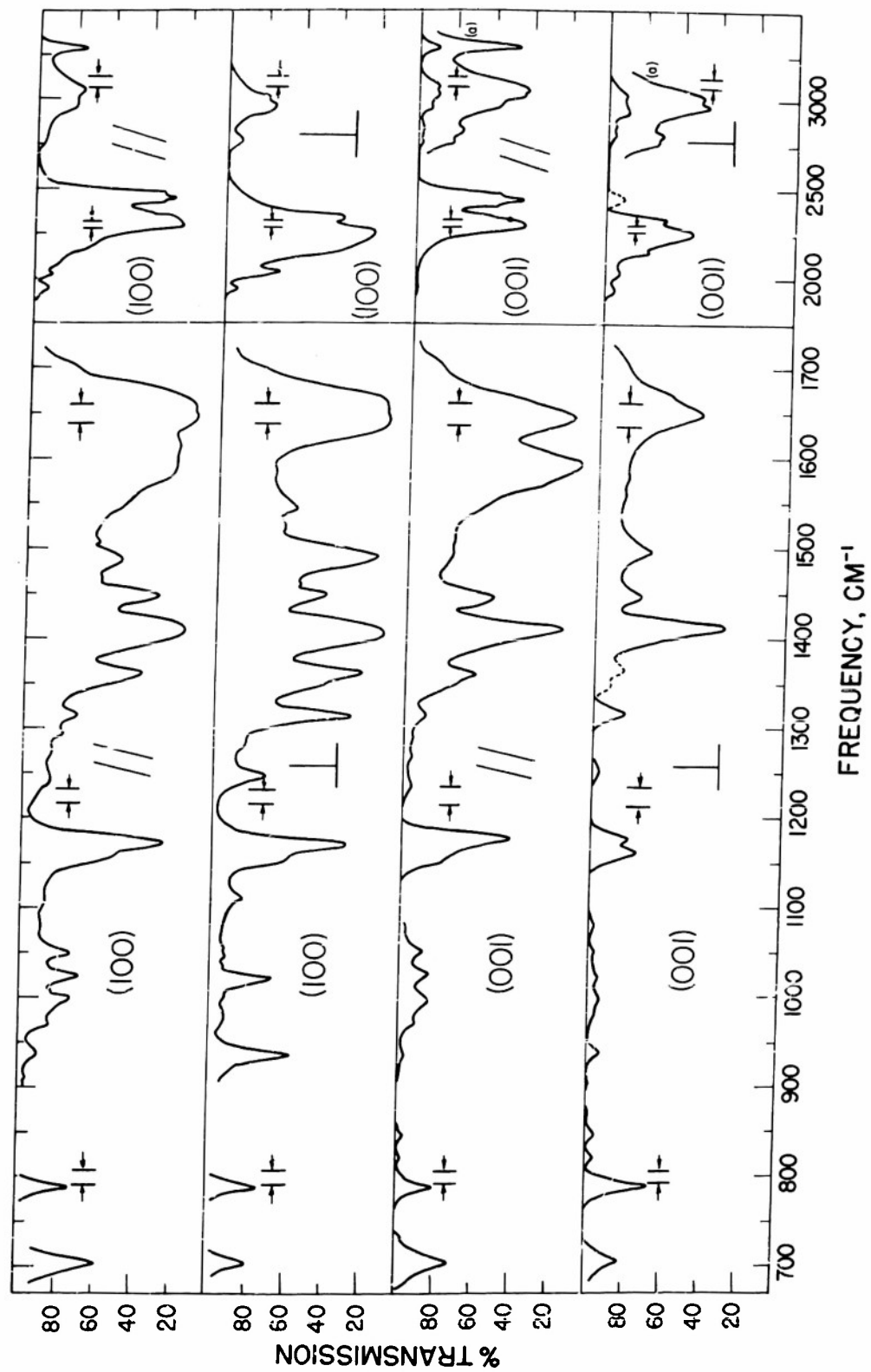


Figure 7

AN INVESTIGATION OF THE SPECTRUM OF SILK FIBROIN

By A. D. E. Pullin and Richard M. Badger

As has been mentioned elsewhere in this and in previous reports, quantitative information regarding the direction of change of dipole moment in the characteristic peptide vibrations has been sought by polarization studies of crystals of simple substances containing the peptide link. As has been shown, these investigations present considerable difficulty both in respect to obtaining sufficiently thin crystals of suitable orientation, and in the correction for reflections in the case of bands which are highly polarized and are consequently of the greatest interest. A more ideal approach would be the examination of polypeptides or proteins of simple, known structure from which information could be obtained which would be useful in the study of unknown structures. The problem of obtaining suitable thin sections appears capable of solution and the "dilution" of the peptide links by side groups, and the relative broadness of the absorption bands reduce the difficulties due to surface reflection. It is true that some new and serious problems present themselves. It may be difficult to obtain specimens in which the crystalline material is perfectly oriented, and a considerable fraction of non crystalline material may be present. Methods for the estimation of the degree of crystallinity have been developed for some synthetic high polymers, but it is doubtful whether they are sufficiently precise, and they have so far not been applied to materials of the sort under discussion.

It seems very improbable that the structure of any polypeptide or protein will be known in the near future with anything like the certainty in detail which is possible for the crystals of substances of moderate molecular weight.

Nevertheless for a few substances plausible structures have been proposed which receive sufficient support from x-ray studies that their tentative use as calibration specimens may perhaps be justified. A particularly suitable substance of this sort is silk fibroin, the structure of which has recently been investigated in this laboratory by Dr. R. E. Marsh,¹ using x-ray methods. Fibroin (*Bombyx mori*) contains a large fraction of the simple amino acids, glycine, alanine, and serine, and a comparatively small amount of residues such as aspartic and glutamic acids, which may be expected to give interference in the 1500-1700 cm^{-1} region. Dr. Marsh has found that the x-ray data indicate a structure of the antiparallel pleated sheet type proposed by Pauling and Corey.² In this structure the C=O and N-H bonds are perpendicular to the fiber axis, which should make it relatively easy to determine deviations in dipole moment change from this direction.

Although the spectrum of silk fibroin has previously been examined by other investigators it seemed desirable to repeat this investigation taking great care to obtain uniform, well oriented sections of suitable thickness for obtaining reliable intensity data. We are greatly indebted to Dr. R. F. Baker and Mr. E. Henderson who prepared these specimens for our use.

The specimens were prepared by the longitudinal sectioning of silk suture which had been embedded in a plastic of suitable hardness. The samples were usually about 1.5 microns in thickness and under the polarizing microscope showed satisfactory uniformity in thickness and absence of holes. A sample was also examined by x-rays to determine whether the orientation was as satisfactory as in material used for the structure studies. A sharp diffraction pattern was obtained, showing that all crystalline material was lined up within a very few degrees.

Qualitative Discussion of the Spectrum

The spectra obtained are in agreement with investigations previously reported^{3,4} except that a somewhat higher degree of polarization was obtained in the $6\ \mu$ region, and the $3\ \mu$ region was examined under much higher dispersion than has previously been employed. The spectrum as a whole is consequently not reproduced but the $6\ \mu$ region is shown in Figure 1 and the $3\ \mu$ region polarized spectrum under high dispersion is shown in Figure 2.

Two features of the spectrum deserve comment. In the region near $10\ \mu$ a band of at least moderate intensity occurs at $1001\ \text{cm}^{-1}$ which is as highly polarized as any in the spectrum, and in the direction parallel to the fiber axis. The dichroic ratio is about 5/1. It consequently must be attributed to well oriented material.

Blout⁵ has recently observed that a band in the region $1000\text{--}1025\ \text{cm}^{-1}$ appears to be characteristic of the glycyl-glycyl linkage, and is absent in peptides not containing this grouping. The chemical evidence on fibroin seems to show that no significant fraction of such groups is present.⁶ Either this evidence is not conclusive or the correlation of the $1000\ \text{cm}^{-1}$ band with the glycyl-glycyl group is not unique. The latter alternative seems the more probable.

The band at $1692\ \text{cm}^{-1}$, occurring as a companion to Amide I is appreciably polarized and consequently is attributed to crystalline material. It presumably has the same significance as the $1688\ \text{cm}^{-1}$ band in a diglycine, which is discussed elsewhere, and its reasonably high intensity is presumably due to the high content of glycine in silk fibroin.

Interpretation of the Polarization Spectrum

It will be noted in Figure 1 that the frequencies of the maxima of the Amide I and IIa bands are significantly different in parallel and perpendicular polarization, and a composite structure of the bands is clearly evident, indicating the presence of both α and β structures according to the criterion of A. Elliott, as he indeed has pointed out.³

In the lower parts of Figure 1 it is shown that the observed curves can be plausibly decomposed into five components. The components at about 1636 and 1528 cm^{-1} , representing the Amide I and IIa bands of the β constituent polarize rather well and must for the greater part originate in oriented material. The components at 1664 and 1560 cm^{-1} do not appear to polarize at all, and must be attributed to a completely disoriented α constituent. The band at 1692 cm^{-1} shows moderate polarization.

It will be noted that the N-H valence band shown in Figure 2 is significantly narrower in perpendicular than in parallel polarization and that the frequency of the maximum is about 2 cm^{-1} higher in the latter case. This may probably be taken as further evidence for the presence of more than one constituent in fibroin. Underlying the N-H band is a much broader absorption which increases in intensity with increasing wave length and is more intense in parallel polarization. We attribute this to the O-H absorption of serine. The polarization is in agreement with the model of Dr. Marsh.

In spite of the high resolution employed it is not possible to make a very accurate estimate of the dichroic ratio in the N-H band because of the uncertainty in making the background correction. This was especially difficult because of the considerable breadth of the band and because the background was

considerably polarized. It was assumed that within the N-H band the background had a smooth trend without maxima and when a plausible correction was made it was found that the absorbance curves could be fitted by the Lorentz formula to 150 cm^{-1} on both sides of the maxima. The dichroic ratios were estimated in two ways. In the first of these it was assumed that the band intensity had reached zero at $\nu_0 \pm 150 \text{ cm}^{-1}$. In the second, the peak heights and total areas were estimated by the assistance of Lorentz curves fitted as described above. The dichroic ratios estimated in these ways from the ratios of peak intensities were 3.0 and 2.75, respectively. The ratios of integral extinction coefficients were 2.34 and 1.85. The lowest of these values, namely 1.85, is probably the most reliable value for the dichroic ratio, though it is by no means certain that the wings of the band follow the Lorentz formula.

Estimation of the Non Crystalline Fraction of Silk Fibroin

As has been shown above, even a qualitative examination of the absorption curves suggests that the silk fibroin used for the infrared studies (and also for the x-ray structure investigations) does not consist entirely of material in a single, organized structure. This material was obtained by stretching of the silk worm gland and there is the possibility that it may be somewhat contaminated internally by sericin, which it does not seem practical to remove. In the spun silk used in chemical studies this material presumably occurs only as a coating on the fiber, which can be removed. However, it has been the belief of several investigators that the purified spun silk contains a considerable fraction of non crystalline material. This opinion has been based on several kinds of experimental observation⁷ which are not entirely in agreement. In particular, an attractive suggestion that valine and other heavier amino acid residues might be contained in a non crystalline portion, constituting perhaps 30% of the total,^{7a,b} seems not to be supported by the results of

Sotue and Konishi. Further work along chemical and other lines is certainly to be desired.

In order to make quantitative use of the dichroic ratios we have observed we shall consequently be obliged to make use of the curves in Figure 1. We shall assume that the β constituent of fibroin is entirely in the form proposed by Marsh and that the transition probabilities for the amide bands of both α and β constituents are the same. From the relative intensities of the two components of the bands we estimate a non crystalline α fraction of 33 per cent, using the Amide I bands, and of 37 per cent, using the Amide IIa bands. Considering the somewhat limited resolving power employed, and other sources of error we cannot claim that the analysis of the data is unique, but the reasonable agreement of the two estimates gives us some confidence and indicates that possible interference from other underlying bands is not too serious.

We have discussed the interpretation of our data with Dr. Marsh and have examined diffraction photographs used by him in the structure investigation. He believes that even a small amount of an oriented α structure could be detected, and evidence for such structure is clearly absent from the photographs. It is uncertain how large a fraction of completely disoriented material could escape detection. This might be fairly large, but it is unlikely that it is much larger than the amount which we have estimated.

Dichroic Ratios and Directions of Change of Dipole Moments

The dichroic ratios and calculated directions of change of dipole moment for the three amide bands are given in Table I. The ratios for the 1635 and 1528 cm^{-1} bands were obtained from the analyses of the absorption curves previously discussed. The values of these ratios are probably not very dependent on the correctness of this analysis in detail, since the overlapping of α and β bands is small. The ratio for the 3292 cm^{-1} band has been corrected for the

non crystalline material.

A discussion of these results and a comparison with other data are given in the following section.

Table I
Dichroic Ratios and Directions of Change of Dipole Moments

Band	(cm ⁻¹)	$I_{\parallel} / I_{\perp}$	Inclination of dipole moment	
			with perpendicular to fiber axis	with C=O direction in amide plane**
Amide I	1635	1/2.6	24°	25°
Amide IIa	1528	2.9 ₅	50.5°	54.5°
N-H stretch	3292	(2.3 ₄ - 1.8 ₅) 4.7* - 2.9*	17.5° - 22.5°	18.5° - 24°

*Corrected for 35 per cent non crystalline material.

**The change in dipole moment is assumed to lie in the amide plane, which in the Marsh model is inclined 20.5° with the fiber axis.

References for Section 4.

1. Reported at the Pasadena Conference on the Structure of Proteins, California Institute of Technology, September 1953. (See J. T. Edsall, Science 119, 302 (1954), and J. C. Kendrew, Nature 173, 57 (1954).
2. L. Pauling and R. B. Corey, Proc. Nat. Acad. Sci., 39, 253 (1953).
3. E. J. Ambrose and A. Elliott, Proc. Roy. Soc. (London) A 206, 206 (1951).
4. M. Goldstein, Ph.D. Thesis, Columbia University, 1950.
5. E. R. Blout and S. G. Linsley, J. Am. Chem. Soc. 74, 1946 (1952).
6. M. Levy and E. Slobodiansky, Cold Spring Harbor Symposia, Quant. Biol., 14, 1113 (1949).
7. See for example:
 - a. K. H. Meyer, M. Fuld, O. Klemm, Helv. Chim. Acta, 23, 1441 (1940).
 - b. B. Drucker and S. G. Smith, Nature 165, 196 (1950).
 - c. Hiroshi Sobue and Hikoichi Konishi, J. Chem. Soc. Japan, Ind. Chem. Sect., 56, 168 (1950).

Captions for Figures of Section 4.

Fig. 1. Polarized spectrum of a thin section of silk fibroin in the 6μ region.

A. shows the spectra observed with light polarized parallel to and perpendicular to the fiber axis; B and C show an interpretation of the complex structure of the absorption bands.

Fig. 2. Polarized spectrum of silk fibroin in the 3300 cm^{-1} region. The spectrum has been corrected for an underlying absorption, as described in the text. The circles and crosses represent the best fit of the Lorentz formula to the observed band shapes.

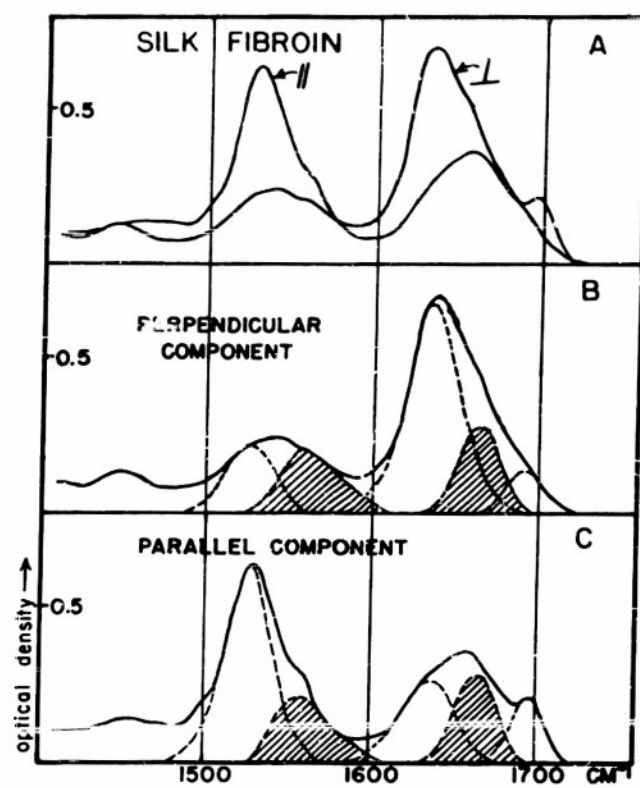


Figure 1

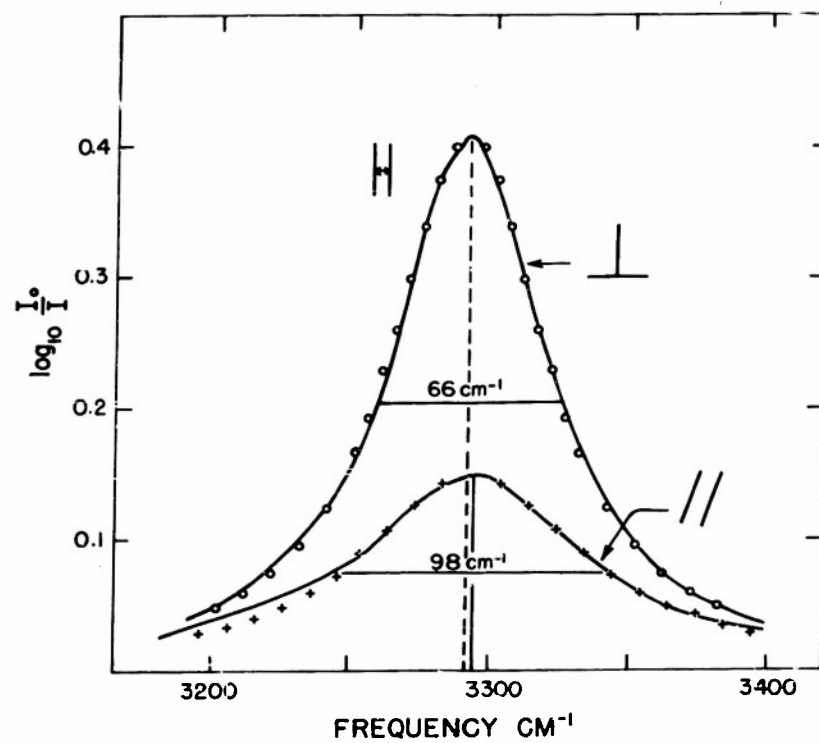


Figure 2

SUMMARY OF INFORMATION RELATING TO THE DIRECTION OF CHANGE OF DIPOLE
MOMENT IN THE CHARACTERISTIC AMIDE AND PEPTIDE VIBRATIONS

A question of considerable interest is the extent to which quantitative infrared polarization data on fibrous proteins or other oriented polypeptides can be useful in determining the configuration of a polypeptide chain. It would certainly be very convenient if the peptide links in a chain could to a first approximation be regarded as acting independently, and the relative transition probabilities for crystal vibrations causing absorption polarized parallel and perpendicular to the fiber axis could be predicted from the appropriate in phase and out of phase combinations of local effects produced by these semi-independent groups. If in addition the local changes in dipole moment produced in a characteristic peptide vibration were always in a definite and invariant direction with respect to a peptide link, polarization data should be of great value in elucidating the structures of systems which have a repeating unit which is not very large. This of course presupposes either that perfectly crystalline and perfectly oriented samples of material are available for study, or that proper allowance for disorientation can be made. Methods such as are used for estimating the crystallinity of cellulose and of synthetic high polymers have not as yet been well developed for proteins, which appear to present more difficult problems.

Unfortunately both theory and experience predict that the ideal conditions mentioned above cannot be expected to hold strictly, although it does not seem to be possible at present to predict the magnitude of the deviations. That they may be rather large is shown, for example, by an observation made in this laboratory.

In crystalline thiourea the molecules sit on sites of lower symmetry than the isolated molecules would presumably possess. Namely the molecules do not lie in a symmetry plane although the heavy atoms appear for all practical purposes to be coplanar. Polarization data indicate that changes in dipole moment may deviate as much as 12° from the molecular plane in vibrations which would be planar in an isolated molecule and are probably not far from planar in the crystal.¹

Though the above example shows that one may find deviations from the simple, naive picture it has nevertheless seemed sensible to make an empirical approach and to compare polarization data from sources as are presently available. The ideal situation would be to take data from simple polypeptides of which the structures have been definitely and unambiguously determined. Unfortunately it can scarcely be said that such exist in the same sense that is true for a number of dipeptides. Nevertheless it seems highly probable that the crystalline portions of some oriented synthetic polypeptides, for example poly- γ benzyl -L- glutamate, do have the α -helix structure² and that the structure of the crystalline portion of silk fibroin is of the pleated sheet type found by R. E. Marsh to be in agreement with x-ray data (see Section 4 of this report.)

We have consequently included data on these two substances in Table I, which gives the direction of the change of dipole moment in the three characteristic peptide vibrations calculated from the polarization data on the assumptions regarding non interaction of the peptide links with their environment which were described above. In each case the direction of the transition moment has been referred to the direction of the C=O bond on the assumption that it lies in the O-C-N plane. The C=O bond was taken as the reference direction in all cases since the peptide hydrogen atom is not located by x-ray methods, and the direction of the N-H bond is not known, and indeed may be uncertain to the extent of several degrees.

In both of the two cases mentioned above the degree of crystallinity of the specimen examined comes in question. The correction made for silk fibroin is discussed in Section 4 of this report. The polarization data for poly- γ - benzyl- L - glutamate are those of Ambrose and Elliott³ and it has been assumed that the specimen was completely crystalline and oriented. This may not be entirely justified though the benzyl-glutamate showed as high dichroism as has been observed in any synthetic polypeptide.

Data on three crystalline substances of moderate molecular weight are included in Table I. Those on acetyl glycine⁴ and α - diglycine⁵, which were examined in this laboratory, relate primarily to the transition moment for the N-H valence vibration, for which the experimental conditions for observation were unusually favorable. Acetyl glycine showed no dichroism in the N-H band when observed from a direction perpendicular to the plane of the peptide link, from which it was concluded that the direction of the transition moment made an angle of 45° with the b axis of the crystal. The observations on α - diglycine are discussed in detail in Section 3. It should be remarked that in both substances the environment of the peptide link is quite different from that in a polypeptide chain, which may restrict the validity of conclusions which may be drawn from the observations. In particular, interaction of the peptide N-H stretch and Amide I vibrations with the stretching and bending motions of the NH_3^+ group of α - diglycine, may be significant. In any case the Amide I band of this substance was overlapped by neighboring bands and the estimation of dichroic ratio was rather uncertain.

The data on acetanilide are due to Bamford and coworkers⁶ and can be accepted only with some reservations. Since thin crystals of the most suitable section were not available, observations were made on a tilted crystal, which tends to enhance errors due to surface reflection. For this reason it seems highly probable that the estimated angle of inclination of the Amide I transi-

tion moment with respect to the C=O bond is a lower limit, and may actually be somewhat larger.

Table I

Inclination of Transition Moment in Peptide

Vibrations with Respect to C=O Bond*

Substance	N-H Valence	Normal Mode	
		Amide I	Amide IIa
1. poly- γ -benzyl- L -glutamate	6°	26°	90°
2. silk fibroin	$18-24^\circ$	25°	54°
3. α -diglycine	6.5°	($56^\circ?$)	($90^\circ?$)
4. acetyl glycine	16°	-	-
5. acetanilide	-	22°	-

*It has been assumed that the direction of the transition moment lies in the O=C-N plane.

It should be mentioned that the dichroism of substances 2, 3 and 4 in Table I was measured in light that was converging to a considerable extent. This should probably cause no error in acetyl glycine owing to the absence of dichroism, as mentioned above. In the investigation of α -diglycine, a study of the effect of stopping down the microilluminator indicated that errors due to non parallel light were small. Nevertheless measurements made in non parallel light must always be suspect since large errors can sometimes arise from causes which are not obvious.⁷ If quantitative measurements of dichroism prove to be of value in structural investigations some effort must be devoted to improving the conditions of observation on the small samples which frequently must be used if the specimens are to be adequately thin and uniform.

Of the three normal modes investigated the Amide I vibration seems to have a transition moment in the most constant direction, which is nearly parallel to the line connecting peptide C and O atoms. Possible reasons for this have been discussed in Section 2. Reasons for a possible deviation in the case of α -diglycine have been given.

It should be mentioned, however, that recent measurements of Elliott⁸ on well oriented nylon suggest a considerably smaller angle, through an accurate estimate could not be made in the absence of independent measurements on the degree of crystallinity.

The moment of the N-H vibration seems never to be in the direction which might be inferred for the N-H bond if one assumes reasonable bond angles, but is always inclined by several degrees toward the extension of the C-N bond. A probable reason for this direction is discussed toward the end of Section 3. One might expect the transition moment to be rather sensitive to the location of the oxygen atom involved in the N-H...O hydrogen bond. In the α helix and in the antiparallel pleated sheet structure the three atoms have been presumed to be at least nearly collinear. In both acetyl glycine and α -diglycine if

the N,H and O atoms are in a straight line the $\text{CH}_2\text{-N-H}$ angle must be considerably larger than is normally to be expected. (A determination of the location of the hydrogen atom by neutron diffraction would be of great interest.) However, no correlation between direction of N-H transition moment and location of the oxygen atom is evident in Table I.

The Amide IIa vibration would seem to be particularly useful in structural investigations but at present it is not possible to make any generalization in its regard. On the experimental side it is probable that interference with overlapping bands is more serious than in the case of the more intense Amide I band. Theoretically one might expect considerable sensitivity to environment since the change in dipole moment results from a combination of effects due to N-H bend and pseudosymmetrical stretch of the O=C-N group.

It is evident that more data must be assembled before definite generalizations can be made and before one can assess the value of polarization data in structural investigations. The applicability of the data on dipeptides and simple amides to the polypeptide problem will in any case be somewhat questionable until proven otherwise and some systematic differences between helical and sheet structures may well be expected. For example, in the molecular N-H or Amide I vibrations which produce the more intense of the two polarized components the peptide groups all move in phase in the α -helix structure. In the pleated sheet structure alternate groups are 180° degrees out of phase. Similarly the molecular modes which give rise to the less intense component are different in the two types of structure. Consequently if there is interaction between adjacent groups the inclination of the local dipoles (with respect to the peptide links) calculated from dichroic ratios may appear to be different in the two structures.

It would be very desirable to have polarization data on some known structures containing the proline residue, since such information might be of

assistance in elucidating the structure of collagen which up until now has presented great difficulty. For reasons discussed in Section 9 it seems probable that the proline residue will not make any contribution in the Amide IIa region, and N-H absorption is obviously absent. These differences from the usual residues constituting the polypeptide chain might well be of considerable assistance in distinguishing the location of proline residues which presumably play a rather unique role.

References for Section 5

1. Robert D. Waldron, Ph.D. Thesis, California Institute of Technology, 1952.
2. L. Pauling, R. B. Corey and H. R. Branson, Proc. Nat. Acad. Sci. (U. S.) 37, 205 (1951).
3. E. J. Ambrose and A. Elliott, Proc. Roy. Soc. (London) A 205, 47 (1951).
4. R. Newman and R. M. Badger, J. Chem. Phys., 19, 1147 (1951).
5. See Section 3 of this report.
6. G. H. Bamford, L. Brown, A. Elliott, W. E. Hanby and I. F. Trotter, Proc. Roy. Soc. (London) A 141, 49 (1953).
7. See R. Newman, J. Chem. Phys., 20, 444 (1952).
8. A. Elliott, Nature 172, 359 (1953).

THE MEASUREMENT OF INFRARED EXTINCTION COEFFICIENTS AND DICHROIC RATIOS OF SOLIDS

By A. D. E. Pullin

The purpose of this communication is to discuss the errors that will arise in the determination of infrared extinction coefficients and dichroic ratios from measurements on solids unless correction is made for the accompanying reflection bands. That reflection bands accompany the strong infrared absorption bands has been known for a long time,¹ and recently there has been renewed interest in this field.^{2,3} Organic crystals as well as ionic crystals may give strong reflection bands. Fig. 1 shows part of the reflection spectrum of a diglycine. This was obtained with radiation incident at 20° on the (001) face of a 1mm. thick crystal with the electric vector polarized perpendicular to the plane of incidence and parallel to the symmetry axis [010] of the crystal, which closely parallels the peptide N-H and C=O directions.⁴

For thin crystals such as are used for transmission measurements reflection effects are still important. Connected also are interference effects within the crystal caused by partial internal reflection. The transmission of thin absorbing films has been discussed theoretically by a number of authors including H. Murman^{5,6,7} who derived an expression for the transmission of radiation incident perpendicularly on a plane parallel sheet of an isotropic absorbing material. The final expression he gives for the transmission (reference 5, middle of p. 743) is too big by a factor of 2,* and should be:

*This can be easily seen by putting $\alpha = \beta = 0$ corresponding to zero thickness of absorber in his expression which then gives a value of 2 instead of 1 for the transmission. Otherwise the expression is correct.

$$\text{Transmission} = T(1) = \frac{8 (n^2 + k^2)}{A \cosh 2\beta + B \sinh 2\beta + A' \cos 2\alpha + B' \sin 2\alpha} \quad (1)$$

$$\text{where } A = 1 + 6n^2 + 2k^2 + n^4 + 2n^2k^2 + k^4$$

$$A' = -1 + 6k^2 + 2n^2 - k^4 - 2n^2k^2 - n^4$$

$$B = 4(n + n^3 + nk^2)$$

$$B' = 4(-k + k^3 + kn^2)$$

$$\alpha = \frac{2\pi n d}{\lambda} \quad \beta = \frac{2\pi k d}{\lambda}$$

d = thickness of layer and λ = wavelength of radiation in vacuo. n and k are the components of the complex refractive index $n - ik$.⁸

In Table I a comparison is made of the true extinction coefficient k with the "apparent" value k' which would be obtained by the use of Lambert's law, $(I/I_0) = \exp - \frac{4\pi k d}{\lambda} = \exp (-\beta)$, with the neglect of surface effects. Columns 4 and 5 of this table give the results of calculations for three sets of n, k values and for a number of thicknesses of absorber. The values $n = 1.47$, $k = 0.4$ are based on published n, k curves for unoriented polyethylene³ (3.43μ band) and the values $n = 1.75$, $k = 2.0$ on similar curves for liquid CCl_4 ^{2a*} (12.8μ band). Reasons will be given later for supposing that the values $n = 2$, $k = 4$ may be approximated by a diglycine and other simple peptides in the 6μ region.

The calculated transmissions $T(1)$ in Table I are absolute values. In practice relative transmission values are usually found, that is points on the spectrum curve outside the region of appreciable absorption are taken as representing 100% transmission and the transmission at the absorption bands calculated therefrom. Values of the actual transmission at points away from absorption bands may be found from (1) by setting $k = 0$ and n at the appropriate value. The choice of n is complicated by the dispersion of the refractive index in the neighborhood of absorption bands.⁹ Relative transmission values were

*Fig. 16 of this reference. The curve labelled K should be labelled K_0 corresponding to our k . I. Simon, private communication.

obtained from the absolute values $T(1)$ column 5 by dividing the latter by the transmission values of the same thickness of material for $k = 0$, and $n = 1.47$ (a) and $n = 1.50$ (b) and (c). For $k = 2$, $n = 1.75$ and $k = 4$, $n = 2$ these were very close to the absolute values $T(1)$. From these relative transmission values an apparent extinction coefficient k'' was obtained in the same way as k' from the $T(1)$ values. The ratio of k''/k is given in column 7, Table I.

The measurement of relative extinction values is of considerable interest in connection with the determination of dichroic ratios. In determining dichroic ratios measurements are made of the ratio of the extinction values for radiation polarized parallel and perpendicular to the symmetry axis of a crystal or fibre. The ratio of the 'parallel' to the 'perpendicular' values of the extinction of the absorption band is usually equated to the square of the cotangent of the angle θ of the direction of the transition moment with the symmetry axis. Since the usual effect of neglect of reflection in the determination of extinction values is to give too high a value, and the ratio of the apparent to the true value tends to increase with increasing extinction values, dichroic ratios obtained directly from observed transmission values should tend to extreme values, that is, to be too great if greater than unity, and too small if less than unity. An approximate idea of the corresponding angular error can probably be obtained from Table I by taking the transmission values corresponding to the pairs of k values (0.4, 2.0), (0.4, 4.0) and (2.0, 4.0) as the transmission values for the two directions of polarization defining a dichroic ratio. If this is done, using relative transmission values and comparing the data for the same value of $\frac{2\pi d}{\lambda}$ in each case, it is found that the errors in the determination of the direction of the transition moment arising from neglect of reflection are small. They are less than 1° for the pairs of k values (2.0, 0.4) and (4.0, 0.4) corresponding to true values of θ of $\sim 5^\circ$ and $\sim 11^\circ$, and less than 3° for the pair of k values (4.0, 2.0) corresponding to a

true value of θ of $\sim 27^\circ$. The errors in the last case are all in the direction suggested above.

These results are probably relevant to current infrared dichroism studies of simple crystalline peptides. Substitution of the n, k values $n = 1.75$, $k = 2$ and $n = 2$, $k = 4$ into the well known expression for the reflection of radiation from a thick isotropic absorbing layer:

$$\% \text{ Intensity reflected} = 100 \cdot \left\{ (n - 1)^2 + k^2 \right\} / \left\{ (n + 1)^2 + k^2 \right\} \dots (2)$$

gives values of 40% and 68% respectively. These are close to the values for the 3μ and 6μ reflection bands in Fig. 1.

It does not seem worthwhile to make more elaborate calculations at the present time, since (1) is strictly applicable only to an isotropic plane parallel sheet of absorber in air (vacuum). Dichroic ratios are a property only of non-isotropic materials and with the thin samples needed for absorption measurements it is not usually possible to insure that the surfaces are plane and parallel. Also in the case of crystals the spectra are often obtained with the crystal adhering to a plate of silver chloride, fluorite etc. for which condition (1) is not applicable.

If the transmission measurements are made with radiation not incident normally on the sample, the reflection will be increased. This is well shown by the curves calculated by I. Simon^{2a} for reflection at 20° and 70° incidence for various n and k values.

In the case of bands caused mainly by hydrogen motion it is possible to avoid reflection difficulties by making the measurements on the residual hydrogen bands from a crystal in which the hydrogen has been almost completely substituted by deuterium.

Table I

<u>n, k,</u>	<u>$2 \pi d/\lambda$</u>	<u>$d(\mu)$</u>	<u>$100 \cdot \exp(-2\beta)$</u>	<u>$T(1)(\%)$</u>	<u>k'/k</u>	<u>k''/k</u>
(a)	0.125	0.068	90.5	87.0	1.45	1.39
	0.250	0.136	81.9	77.0	1.41	1.31
n = 1.47	0.500	0.273	67.0	63.3	1.31	1.14
k = 0.4	1.000	0.545	44.9	43.8	1.21	1.03
$\lambda = 3.43\mu$	1.500	0.818	30.1	32.2	1.02	0.94
	2.000	1.090	20.2	19.6	1.02	1.02
(b)	0.05	0.102	81.9	72.2	1.63	1.63
	0.0625	0.128	77.9	67.0	1.60	1.60
n = 1.75	0.100	0.204	67.0	54.3	1.53	1.52
k = 2.0	0.125	0.255	60.7	67.7	1.48	1.47
$\lambda = 12.8\mu$	0.250	0.510	36.8	26.9	1.31	1.29
	0.500	1.02	13.5	10.3	1.13	1.08
(c)	0.05	0.048	67.0	48.1	1.83	1.83
	0.0625	0.060	60.7	41.0	1.78	1.78
n = 2.0	0.100	0.095	44.9	26.4	1.66	1.66
k = 4.0	0.125	0.119	36.8	20.3	1.59	1.59
$\lambda = 6.0\mu$	0.250	0.239	13.5	6.6	1.36	1.35

References for Section 6

1. C. Shaffer and F. Matossi, Das Ultrarote Spektrum, Julius Springer, Berlin 1930. Lithoprinted, Edward Brothers, Ann Arbor, 1943.
- 2a. I. Simon, J. Opt. Soc. Am., 41, 336 (1951).
- 2b. I. Simon, J. Chem. Phys., 21, 23 (1953).
3. T. S. Robinson, Proc. Phys. Soc. (London) 65B, 911 (1952).
4. Figures showing unit cell projections of a diglycine are included in Section 3 of this report.
5. H. Marnett, Z. f. Physik, 54, 741 (1929).
6. A. W. Crook, J. Opt. Soc. Am., 38, 954 (1948).
7. W. H. Brattain and H. B. Briggs, Phys. Rev., 75, 1705 (1948).
8. Some writers express the complex refractive index as $n(1 - ik) = n - ik_0$.
9. A concise account of the classical theory of dispersion is given by A. A. Korff and G. Breit, Rev. Mod. Phys., 4, 471 (1932).

Caption for Figure for Section 6.

Fig. 1. Portions of the reflection spectrum of a crystal of α diglycine 1 mm thick. Angle of incidence was 20° on a (001) face with electric vector of incident radiation perpendicular to the plane of incidence and parallel to symmetry axis of crystal.

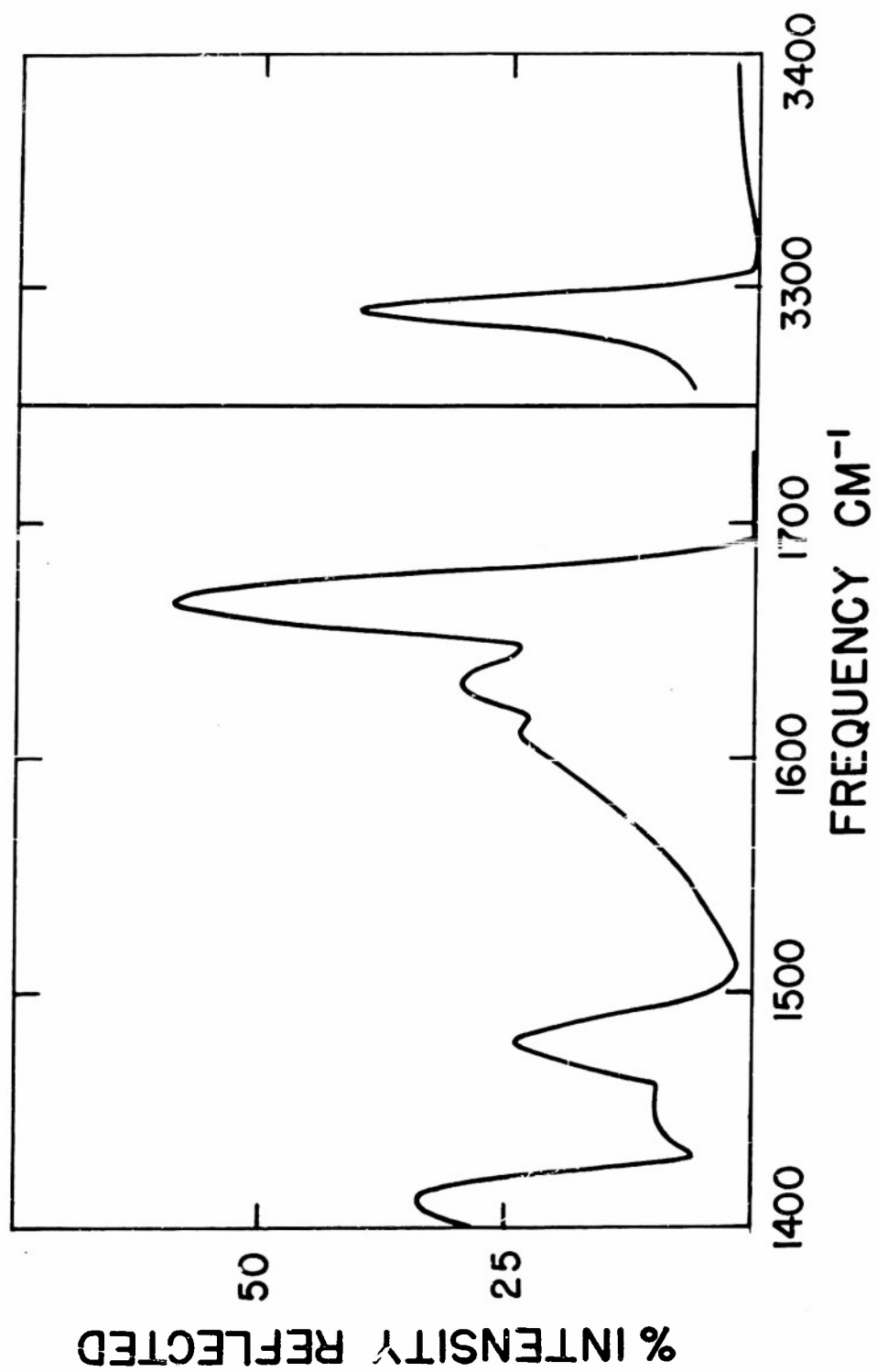


Figure 1

STRUCTURE IN THE 3μ REGION OF THE SPECTRA
OF ASSOCIATED AMIDES AND CARBOXYLIC ACIDS

By Richard M. Badger and Hector Rubalcava

In the 3μ region of the spectra of a number of substances associated by hydrogen bonding, either in solution or in the crystalline state, it is common to find considerably more structure than can directly be attributed to N-H or O-H fundamentals. Often this structure appears to be overlaid on a broad continuum; sometimes it may be somewhat detached from the major absorption region. The intensity of the excess components may be considerably greater than is ordinarily to be expected for overtone and combination bands, which are, indeed, very weak in the monomer spectrum. This has given rise to considerable speculation, but no satisfactory general explanation appears to have been given though one is needed for the complete interpretation of the spectra of peptides and related substances of current interest.

An explanation of the spectra of amides and carboxylic acids in solution has recently been proposed¹ which we have found somewhat obscure, but which attributes the structure of the spectrum to combination tones, chiefly difference tones, of the N-H or O-H fundamentals with low frequency "intermolecular" vibrations. We believe that this suggestion must be rejected for several compelling reasons. In the first place it does not account for the fact that the structure of the spectra of a homologous series is largely independent of molecular weight, on which the frequency of the intermolecular vibrations must depend. Three quite different carboxylic acids have been shown to exhibit very similar features in their association spectra², and we have observed that in a given solvent acetamide, propionamide and butyramide give spectra which are virtually indistinguishable in the 3μ region. Secondly, because of the very low frequency of the

intermolecular vibrations, the sum and difference tones should be of comparable intensity. No symmetry is observed, however, in the structure of the absorption region.

It seems very probable that the overall breadth of the absorption region is largely attributable to a band sequence in the high frequency OH or NH stretching fundamental, since it is easily accounted for with a reasonable assumption regarding the interaction between low and high frequency vibrations. Though combination tones of the sort under discussion undoubtedly make some contribution, they will likewise appear as sequences of comparable breadth and should scarcely give rise to any discernible structure except at very low temperatures.

It is consequently necessary to find causes for structural features of fairly high intensity and less breadth than the envelope of the N-H or O-H sequence. These must be independent of molecular weight, but be characteristic of the functional groups responsible for association.

We offer the following suggestion. Both carboxyl and amide groups have several fundamental frequencies of which the first overtones or binary combinations lie in the $3\ \mu$ region. They are considerably shifted on hydrogen bonding, which suggests that one may expect a rather large interaction with the N-H or O-H vibration. It should be pointed out that in both carboxyl and amide groups two resonating structures make significant contributions to the ground state, and that their relative importance is rather dependent on hydrogen bonding. It is consequently plausible that the overtones and combinations should borrow intensity when the high frequency fundamental is shifted into their neighborhood and experiences the enormous gain in intensity which results from hydrogen bonding.³ The combinations and overtones mentioned will naturally also occur as sequences, but these will be more compact than those of the NH or OH fundamentals for reasons which should be obvious. These sequences may of course be repeated in combination with low frequency vibrations giving rise to fine structure

as was reported in the spectrum of trichloroacetic acid.⁴

In the present connection we are especially concerned with substances which associate to cyclic dimers. We have recently shown that the unsubstituted amides show this behavior in CCl_4 solution⁵ and that the dimers may be regarded as having a center of symmetry, at least as regards ring vibrations. Consequently every fundamental of the monomer exists in the dimer as both odd and even vibrations of which only the former is infrared active. Each first overtone may appear only singly, but a binary combination may appear as a doublet. This is probably true also of the dimer acids.

In the association spectra of three acids major features have been observed at about 2560 cm^{-1} , 2700 cm^{-1} (a doublet in acetic acid) and 3000 cm^{-1} ² (a doublet in each case). It seems plausible to assign the first two to $2\nu(\text{C-O})$ and $2\nu(\text{C-H bend})$. The last region may include fundamental CH frequencies, but since it appears in trichloroacetic acid a large contribution by $\nu(\text{C-O}) +$

$\nu(\text{C=O})$ seems very probable. The spectra of the acid dimers have not been analyzed in detail but in acetic acid⁶ it seems certain that $\nu(\text{C-O})$ and $\nu(\text{C=O})$ lie at about 1282 and 1739 cm^{-1} respectively. The region about 1400 cm^{-1} probably contains both a methyl deformation fundamental and the OH bend since we have observed a strong band at this location in trichloroacetic acid. The overtone of a methyl deformation may account for the extra component which is found near 2700 cm^{-1} in acetic acid, but is absent in benzoic and trichloroacetic acids.

In carbon tetrachloride solution the spectra of the dimers of the unsubstituted amides exhibit two strong components (P_1 and P_5) as shown in Figure 1. The band P_5 at 3505 cm^{-1} is attributable to the N-H hydrogens not engaged in hydrogen bonds⁵ and is very narrow, but the hydrogen bonded N-H absorption with maximum at 3172 cm^{-1} (labelled P_1) is very broad and appears to extend at least to the monomer band at 3422 cm^{-1} . It is overlaid by three weaker components, P_2 , P_3 and P_4 at 3239 , 3307 and 3359 cm^{-1} . It is plausible to assign these to

$2 \nu(\text{N-H}_2 \text{ scissors}), \nu(\text{NH}_2 \text{ scissors}) + \nu(\text{C=O}),$ and $2 \nu(\text{C=O}).$ ⁷ As shown in Figure 2 the active carbonyl vibration of the dimer in CCl_4 solution lies at about 1696 cm^{-1} . The NH_2 scissors fundamental is not comfortably observable because of solvent interference, but may be expected to lie slightly above the frequency observed in chloroform solution.

In solvents of higher dielectric constant several interesting changes occur, but a simple interpretation may be impossible since the broadening of the P_5 polymer band at higher concentration with definite indications of a second component suggests the presence of several species.⁸ In CCl_4 this band is very narrow up to saturation. The distribution of intensity in the polymer bands is considerably different, indeed the polymer spectrum in chloroform is intermediate between that found in CCl_4 and in the solid state.

Though the structure of crystalline propionamide has not been determined, its spectrum, shown in Figure 1, is practically identical with that of solid acetamide which has been investigated.⁹ In the latter crystal all N-H hydrogens are hydrogen bonded and both symmetric and antisymmetric NH valence vibrations can be active. The two major absorptions below 3μ are probably to be identified with these modes, while the intermediate absorption may well be attributed to the overtone of the NH_2 scissors which appears to be of rather high frequency in the crystal. (We were not able to resolve this band from the broad C=O or Amide I band.)

In solvents of higher dielectric constant there is a general tendency for all frequencies to be decreased somewhat, the percentage shift in overtones being approximately the same as for fundamentals. The shift may be expected to be larger the more intense the fundamental. There is, however, a notable exception to the general direction of shift, namely the fundamental valence frequencies of hydrogen bonded N-H increase with increase in dielectric constant. We suggest that these facts may be very useful in distinguishing fundamental

from overtone and combination bands in the N-H valence region.

As may be seen in Fig. 1, increase in dielectric constant shifts P_1 upwards though all other bands appear to decrease in frequency, although the behavior of P_2 is obscured by overlapping with P_1 . The shifts of P_3 and P_4 seem to be in accord with the interpretation given above, when one considers the interaction between the various upper levels involved. The increased interaction which results from the shifts of frequencies in solvents of higher dielectric constant, may be the explanation for the increase in intensity of P_4 , and possibly also of P_3 . However, we hesitate to attempt a complete interpretation of the spectra in any of the solvents except carbon tetrachloride since in the other solvents the presence of polymers higher than dimers seems possible. The breadth of the P_5 band in chloroform and a suggestion of doubling at high concentrations may well result from the presence of several species. If linear polymers are present in these solvents an absorption is to be expected around 3350 cm^{-1} which may account for the intensity of P_4 .

The substituted amides appear to associate at least predominantly as linear polymers with N-H absorption occurring at about 3360 cm^{-1} in the dimers and shifting to lower frequency with increasing chain length.⁵ A minor absorption region which centers at about 3080 cm^{-1} is of particular interest since it appears to correspond to an absorption commonly found in polypeptides which has sometimes been described as an N-H frequency.¹⁰ In the present instance its interpretation as a fundamental appears to be excluded since this band appears in the earliest stages of association and grows in intensity without significant shift with increasing concentration. In the dimer only one valence frequency of hydrogen bonded N-H seems possible. We believe that it is plausible to attribute this band to the overtone of the Amide IIa fundamental at 1540 cm^{-1} which is characteristic of N-substituted amides. The polarization of the 3080 cm^{-1} band in oriented solid materials is discussed in the section relating to the normal modes of the amides.

It may be recalled that a similar situation occurs in cyanuric acid¹¹ where a doublet satellite accompanies the lower frequency N-H stretching fundamental and shows similar polarization. This satellite is reasonably to be attributed to the overtone of 1470 cm^{-1} which appears to be a ring vibration having some relation to the Amide IIa fundamental of the non cyclic amides. The reason for the doubling is not apparent though it is not in violation of any exclusion rule when one considers the vibrations of the unit cell as a whole.

References for Section 7

1. M. Davies and J. C. Evans, J. Chem. Phys., 20 342 (1952).
2. A. M. Buswell, W. H. Rodebush and M. F. Foy, J. Am. Chem. Soc., 60, 2339 (1938).
3. A strong activation by association seems to occur even in some cases where the interaction between the OH vibration and another frequency is probably less than in the examples under discussion. It is very possible that the band observed at 10900-11100 cm^{-1} in several associated alcohols (see R. M. Badger and S. M. Bauer, J. Chem. Phys., 5, 839 (1937)) is the third overtone of OH activated by the second overtone of OH. No similar band has been reported in the other harmonics.
4. M. M. Davies, Trans. Faraday Soc., 36 1141 (1940).
5. R. M. Badger and H. Rubalcava, Proc. Nat. Acad. Sci. (U. S.) 40, 12 (1954).
6. R. C. Herman and R. Hofstadter, J. Chem. Phys., 6, 534 (1938).
7. What are here called the C=O and NH_2 scissors vibrations are elsewhere in this report designated as Amide I and Amide IIa, respectively.
8. The distribution experiments of M. Davies and H. E. Hallam on acetamide (Trans. Faraday Soc., 47, 1170 (1951)) indicate the presence of dimers, trimers and higher polymers in chloroform solution, with trimers predominating at higher concentrations. These results must be accepted with some reservation until possible effects of water have been investigated. The association of phenol in CCl_4 is markedly affected by water and the predominant polymer species appears to contain two molecules of phenol and one of water.
9. F. Senti and D. Harker, J. Am. Chem. Soc., 62, 2008 (1940).

10. For literature references see the discussion by R. D. B. Fraser, page 50, Progress in Biophysics, Vol. 3, Pergamon Press Ltd., London, 1953.
11. R. Newman and R. M. Badger, J. Am. Chem. Soc., 74, 3545 (1952).

Caption for Figures of Section 7.

Fig. 1. The spectrum of propianamide in three solvents and in the solid state in the $3\ \mu$ region.

Fig. 2. Spectrum of propianamide in two solvents of the $6\ \mu$ region.

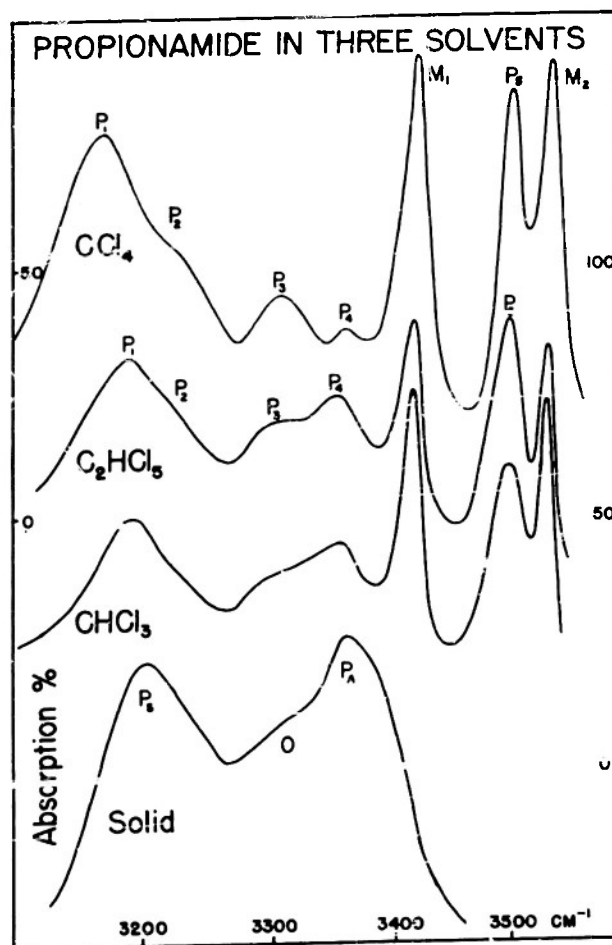


Figure 1

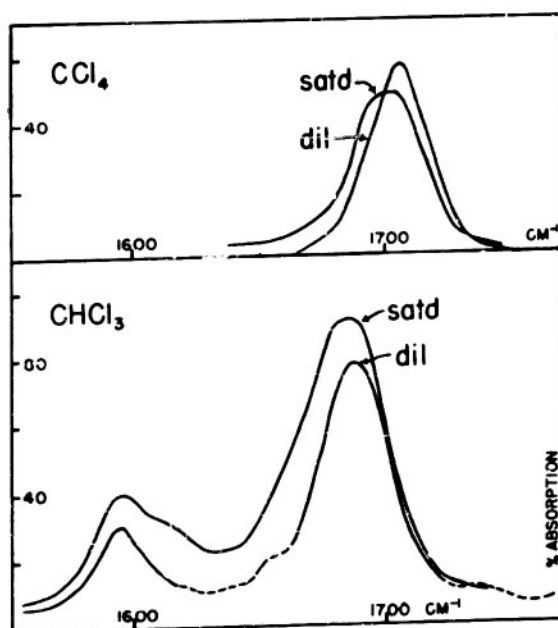


Figure 2

The Infrared Spectrum and Structure of Collagen*†

RICHARD M. BADGER AND A. D. E. PULLIN
Gates and Crellin Laboratories of Chemistry,
California Institute of Technology, Pasadena, California
(Received March 25, 1954)

IN view of the present interest in the structure of collagen it may be useful to point out that spectroscopic observations may greatly assist one in discarding preliminary structural models, though they do not, by themselves, lead to a solution of the problem. For example, the question has arisen as to whether the collagen structure may be entirely, or even in considerable part, based on peptide links with the *cis*-configuration.^{1,2} From existing spectroscopic evidence this seems very unlikely. It is even more improbable that the structure contains a significant fraction of such groups hydrogen bonded to each other so as to form the eight-membered rings required by the Crick model.³ The first conclusion is based on the intensity of absorption in the amide II,³ or 1540 cm^{-1} band, which appears to be quite normal in collagen and gelatin. This fundamental, characteristic of *trans* amide groups, appears to correspond to the higher frequency member of a pair of modes resulting primarily from the interaction of a symmetrical vibration of the $\text{O}=\text{C}-\text{N}$ group and a $\text{N}-\text{H}$ bend.⁴ In the *cis*-configuration this interaction becomes negligible for geometrical reasons, and the amide II frequency should consequently decrease considerably, probably with the loss of considerable intensity. The plausibility of this conclusion is attested by the fact that normal amide II absorption is quite absent in the spectra of cyclic amides, which offer the only certain examples of the *cis*-configuration.⁵

We have previously pointed out that the eight-membered ring configuration is characterized by an abnormally low $\text{N}-\text{H}$ valence frequency.⁶ This fundamental in collagen, however, appears to be quite normal.

In regard to positive requirements for a collagen model, we present polarization data (Fig. 1) recently obtained by us which are considerably superior to results previously reported on collagen,⁷ though they are similar to observations on stretched gelatin.⁸ They were obtained on 2-micron sections of kangaroo tail dried under tension.⁹ Exploration of the sample with a polariz-

ing optical microscope indicated a root mean square disorientation of about 9° , though this is certainly not conclusive. This degree of disorder would not materially affect the polarization spectra, but some allowance must be made for other factors which minimize the dichroism. These include the presence of some amide absorption other than that of the peptide links, which very probably makes a significant unpolarized contribution in the regions of interest.

Even a qualitative use of the polarization data, particularly of the positive dichroism of amide II, excludes the possibility of some types of model. Their quantitative application requires a knowledge of the direction of the change in dipole in the various fundamental vibrations. Information on this point is at present inadequate and is difficult to obtain, but from available evidence and from plausible considerations we have been led to the following conclusions. In the $\text{N}-\text{H}$ valence vibration the direction of change of moment is not quite parallel to the $\text{N}-\text{H}$ bond, but is inclined by a maximum of 8° toward the projection of the $\text{C}-\text{N}$ peptide bond. In the amide I vibration the direction is not parallel to the $\text{C}=\text{O}$ bond, but is even more nearly parallel to the $\text{O}-\text{N}$ direction than is suggested by Price and Fraser.⁴

There is considerable evidence which suggests that the amide II vibration owes its activity largely to the contribution of the $\text{N}-\text{H}$ bend, though its frequency is primarily determined by the $\text{O}=\text{C}-\text{N}$ group. If this is the case one should expect the fundamental to be polarized nearly perpendicular to the $\text{N}-\text{H}$ bond, and the first overtone nearly parallel to this bond. This interpretation is supported by the observed polarization of the band near 3080 cm^{-1} , generally found in peptides and N -substituted amides, which is plausibly assigned to the overtone of amide II.

In any quantitative application of the polarization data it must be remembered that for peptide links involving proline nitrogen, absorption will be absent not only in the $\text{N}-\text{H}$ valence region but presumably also in the amide II region. The latter statement is supported by the absence of such absorption in N,N -disubstituted amides, especially in N -acetyl piperidine.⁵

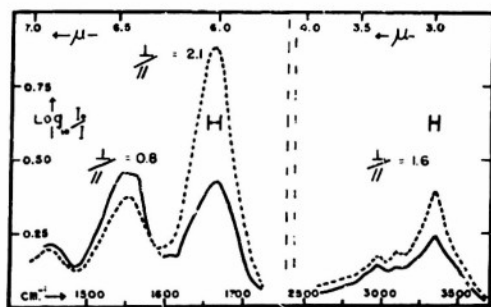


Fig. 1. Polarized infrared spectrum of kangaroo tail tendon (section approximately 2μ thick). Dichroic ratios at band maxima are indicated.

* Contribution No. 1890 from the Gates and Crellin Laboratories.

† Based on investigations supported by the U. S. Office of Naval Research, Contract N6-ori-102, VI.

¹ Pauling, Corey, and Branson, *Proc. Natl. Acad. Sci. U. S. A.* **37**, 272 (1951).

² F. H. C. Crick, *J. Chem. Phys.* **22**, 347 (1954).

³ We follow the nomenclature of Randall, Fowler, Fuson, and Dangle, *Infrared Determination of Organic Structures* (D. Van Nostrand Company, Inc., New York, 1949).

⁴ This interpretation was suggested by W. C. Price and R. D. B. Fraser, *Nature* **170**, 490 (1952); *Proc. Roy. Soc. (London)* **B141**, 66 (1953); and has been confirmed and elaborated by us by the use of a mechanical vibrating model.

⁵ H. Lenormant, *Bull. Soc. Chim.* **15**, 33 (1948).

⁶ R. M. Badger and H. Rubalcava, *Proc. Natl. Acad. Sci. U. S. A.* **40**, 12 (1954).

⁷ Martin Goldstein, Ph.D. thesis, Columbia University (1950).

⁸ E. J. Ambrose and A. Elliott, *Proc. Roy. Soc. (London)* **206**, 706 (1951).

⁹ We are greatly indebted to Dr. R. F. Baker for the preparation of these specimens.

Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES,
Vol. 40, No. 1, pp. 12-17. January, 1951

*THE INFRARED ASSOCIATION SPECTRA OF AMIDES IN SOLUTION
AND THEIR RELATION TO THE SPECTRA OF POLYPEPTIDES**

BY RICHARD M. BADGER AND HECTOR RUBALCAVA

GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated November 10, 1953

The infrared spectra of amides in solution have been the subject of several previous investigations.¹ Nevertheless, in connection with a systematic study of peptides and related substances, it has seemed desirable to make a quantitative reinvestigation of some of these spectra with the greatly improved instrumental facilities now available. The grating spectrometer employed was used under conditions such that water vapor lines separated by 3 cm.⁻¹ were well resolved so that no correction need be made for slit width in intensity measurements. Great precautions were taken in stray light corrections.

In carbon tetrachloride solution acetamide, propionamide, and butyramide exhibit virtually identical spectra in the N—H valence region, from 3100 to 3600 cm^{-1} . A typical example is shown in figure 1. Seven components are distinguishable, the detailed explanation of which will be given in another place. Two of these M_1 and M_2 , with width about 20 cm^{-1} at half peak intensity, are attributable to the monomer, the remainder to associated molecules. The major polymer band, P_1 , undoubtedly to be attributed to hydrogen bonded N—H, is very broad and intense with maximum at about 3172 cm^{-1} .

In the case of propionamide we have shown that the five polymer bands maintain constant relative intensities within the entire available concentration range. This

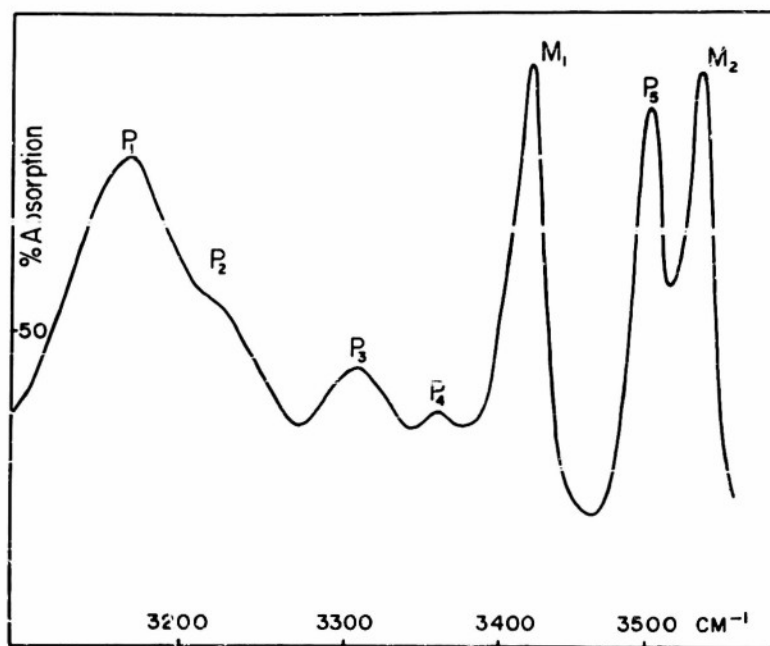


FIGURE 1

The infrared spectrum of propionamide in CCl_4 solution in the 3μ region. Path length 2 cm; concentration approximately 0.004*F*.

strongly suggests the presence of only one polymer species. A quantitative consideration of the dependence of intensity on concentration showed that polymers higher than the dimer can make only a small contribution at the highest concentrations, and a completely satisfactory fit to the data is obtained on the assumption of simple dimerization. This may be seen in figure 2, where the curve corresponds to an association constant, $K_2 = 46.5 \text{ moles}^{-1} \text{ liter}$.

The structure of the spectrum and the intensity measurements lead to definite conclusions regarding the structure of the dimer. In figure 3 it is shown that with increasing concentration the intensities of the dimer bands increase with the squares of the intensities of the narrow bands, M_1 and M_2 . Evidently the latter bands are due exclusively to monomer; the dimer has no free NH_2 end-group. The extreme

narrowness of the polymer band P_6 indicates that it is due to the vibration of N—H not involved in a hydrogen bond. Its frequency is in the range to be expected for the "free" hydrogen of an NH_2 group of which the second hydrogen is engaged in such a bond. Since the dimer has no NH_2 end-group it is difficult to imagine that it has only one such hydrogen, and the intensity of P_6 qualitatively supports the belief that there are two. However, one of the two vibrations involving these two atoms appears to be inactive since P_6 shows no evidence of splitting, but on the contrary is quite as narrow as the dimer bands.

This evidence, together with the dissociation energy mentioned below, makes it difficult to escape from the conclusion that the dimer is cyclic with an eight-membered ring closed by two hydrogen bonds. If the amide group is planar as now

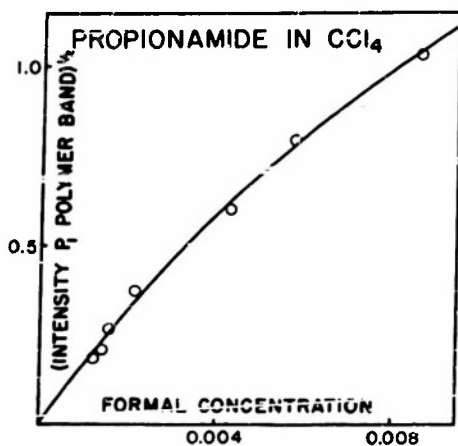


FIGURE 2

Plot of the square root of the optical density at the maximum of the P_1 band, versus concentration (path = 2.54 cm.). The curve corresponds to an association constant $K_2 = 46.5 \text{ moles}^{-1} \text{ liter}$.

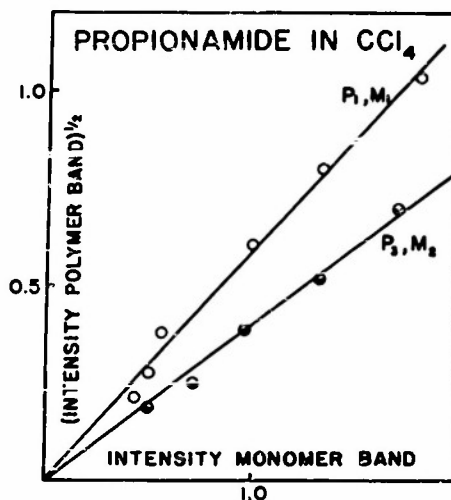


FIGURE 3

Plots of the square root of the optical density at the maximum of a polymer band versus optical density at maximum of monomer band at several concentrations.

seems to be generally admitted, it almost certainly follows from the above that the dimer ring is also planar. The same eight-membered ring configuration closed by two hydrogen bonds has been found in crystalline diketopiperazine² and cyanuric acid.^{3, 4}

The association energy of the dimer of propionamide has been estimated from the temperature coefficient of the peak intensity of the dimer band P_1 . Measurements were made at three temperatures and at two concentrations in a 0.5-inch cell. Figure 4 shows, in effect, plots of $\log K_2$ versus $1/T$. In actual fact the ordinate of a point on the plot is the reciprocal of the concentration which would be required at 25° to give the same degree of association as that observed at T . These reciprocal concentrations are obviously proportional to K_2 . They were estimated from a smoothed plot of the experimental data at 25° .

The best value for the enthalpy of association was found to be $\Delta H = -7850 \pm 220$ cal. mole⁻¹, or -3925 ± 110 cal. per hydrogen bond. The best value for the association constant at approximately 25° is $K_2 = 45 \pm 6$ moles⁻¹ liter which corresponds to $\Delta F = -2250 \pm 80$ cal. mole⁻¹.

In the ring structures joined by two hydrogen bonds mentioned above, the N—H and C=O bonds of a given molecule are *cis* with respect to each other. This *cis* arrangement has, until now, not been demonstrated to exist in any peptide but it has perhaps not been entirely clear whether its absence is due to packing or other steric requirements or to an inherent lower stability than that of the *trans* configuration. It therefore seemed worth while to investigate whether N-methyl formamide is able to associate in the same fashion as the unsubstituted amides, since in this molecule steric interference should be at a minimum. There is no very obvious reason why the dimers of the isomeric acetamide and N-methyl formamide should not be quite similar; in fact they are quite unlike.

The heavier substituted amides evidently associate in quite a different fashion from the unsubstituted compounds. A representative spectrum is shown in the lower part of figure 5. At lower concentrations absorption at 3172 cm.⁻¹ is negligible, though in addition to the monomer band at 3460 cm.⁻¹ a strong absorption centered at 3360 cm.⁻¹ gives evidence of polymer, presumably the dimer. With increasing concentration a succession of new components, evidently due to higher polymers, make their appearance at progressively lower frequencies. This decrease in frequency suggests that the hydrogen bond is stabilized as the size of the polymer increases. At an intermediate concentration the maximum of absorption is at the frequency generally characteristic of dipeptides and polypeptides. From this it is reasonable to conclude that the monomer units are linked in chains in a fashion similar to that in which the peptide groups are linked in crystalline α -diglycine.⁷ This is supported by dielectric constant measurements made on several substituted amides which have shown the presence of chain polymers.^{8, 9}

A broad absorption at about 3090 cm.⁻¹ is particularly interesting since it appears to correspond to a minor absorption commonly found in the spectra of polypeptides, where it has sometimes been described as an N—H frequency. This interpretation appears very unlikely since this band makes its appearance at the earliest stages of association. An alternative explanation will be discussed in another place.

The available concentration range for N-methyl formamide is limited by its moderate solubility, but in the N—H region its spectrum seems to be entirely

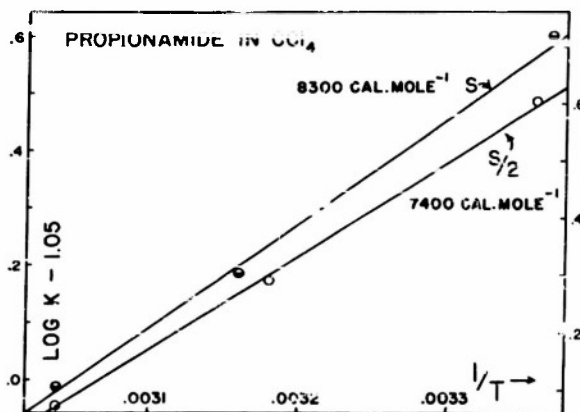


FIGURE 4

Plots of $\log K_2$ versus reciprocal temperature. The symbols *S* and *S/2* refer to solutions which were saturated, or half saturated at 18°.

similar to those of higher substituted amides. One is consequently led to the conclusion that even in this case the *cis* form of the molecule does not exist and that each *trans* monomer is stabilized by an amount very considerably in excess of half the gain which would result from the formation of a second hydrogen bond in closing the dimer ring.

It is of some interest that the N—H frequency of the dimers of the unsubstituted amides is approximately 160 cm.^{-1} lower than is found in those of the N-substituted compounds. We believe that this is not to be attributed to a difference between *cis* and *trans* configurations, but is rather a consequence of the eight-membered ring structure discussed above. Similar differences are found in the crystalline cyclic amides where the *cis* arrangement exists of necessity. In cyanuric acid two-thirds

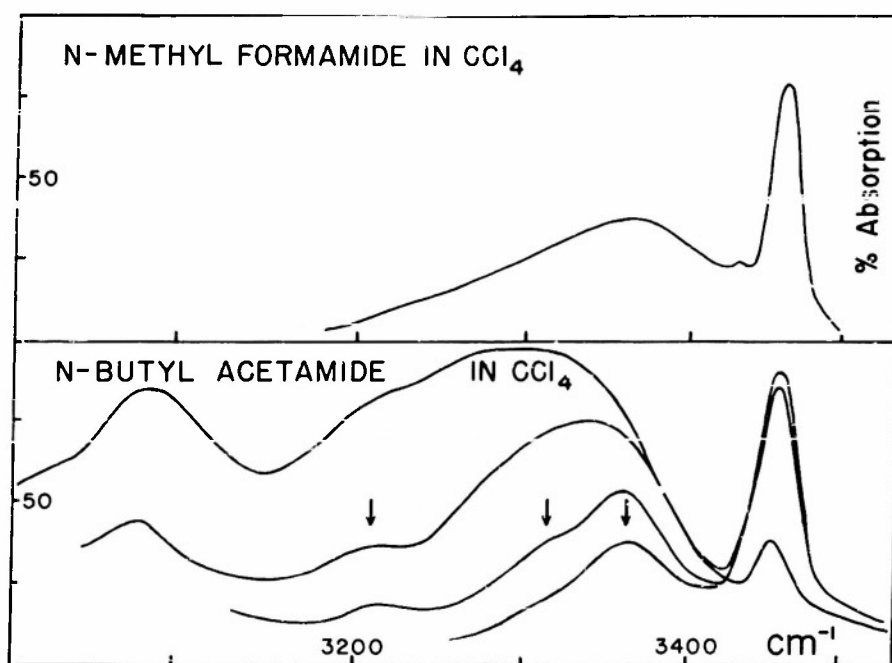


FIGURE 5

The spectra of two N-substituted amides in the 3μ region.

of the hydrogen bonds close eight-membered rings and the corresponding N—H frequency is 3060 cm.^{-1} . The frequency of the remaining N—H groups is 150 cm.^{-1} higher.⁶ In diketopiperazine where all N—H groups are included in rings the N—H absorption is centered slightly above 3000 cm.^{-1} ,⁶ while in parabanic acid where such rings are absent, it lies around 3200 cm.^{-1} .¹⁰ The very low N—H frequency in all these three compounds is possibly the result of stabilization by the many resonating structures which contribute to the ground state of the crystal.

At present there seems little evidence for believing that a *cis* arrangement of N—H and C=O bonds will necessarily result in a low N—H frequency.

* Contribution No. 1859 from the Gates and Crellin Laboratories. The investigations reported were supported by the Office of Naval Research under Contract N 6-ori-102, VI.

- ¹ See, for example, (a) Buswell, A. M., Rodebush, W. H., and Roy, M. F., *J. Am. Chem. Soc.*, **60**, 2444 (1938). (b) Richards, R. E., and Thompson, H. W., *J. Chem. Soc.*, **1947**, 1248. (c) Davies, M., and Hallam, H. E., *Trans. Faraday Soc.*, **47**, 1170 (1951).
- ² Corey, R. B., *J. Am. Chem. Soc.*, **60**, 1598 (1938).
- ³ Wiebenga, E. H., and Moerman, N. F., *Z. Krist.*, **99**, 217 (1938).
- ⁴ Wiebenga, E. H., *J. Am. Chem. Soc.*, **74**, 6156 (1952).
- ⁵ Newman, R., and Badger, R. M., *J. Chem. Phys.*, **19**, 1147 (1951).
- ⁶ Newman, R., and Badger, R. M., *J. Am. Chem. Soc.*, **74**, 3545 (1952).
- ⁷ Hughes, E. W., private communication.
- ⁸ Mizushima, S., Simanouti, T., Nagakura, S., Kuratani, K., Tsuboi, M., Baba, H., and Fujioka, O., *J. Am. Chem. Soc.*, **72**, 3490 (1950).
- ⁹ Hobbs, M. E., private communication.
- ¹⁰ Unpublished investigation.
-

Armed Services Technical Information Agency

AD

44933

NOTICE: WHEN GOVERNMENT OR OTHER DRAWINGS, SPECIFICATIONS OR OTHER DATA ARE USED FOR ANY PURPOSE OTHER THAN IN CONNECTION WITH A DEFINITELY RELATED GOVERNMENT PROCUREMENT OPERATION, THE U. S. GOVERNMENT THEREBY INCURS NO RESPONSIBILITY, NOR ANY OBLIGATION WHATSOEVER; AND THE FACT THAT THE GOVERNMENT MAY HAVE FORMULATED, FURNISHED, OR IN ANY WAY SUPPLIED THE SAID DRAWINGS, SPECIFICATIONS, OR OTHER DATA IS NOT TO BE REGARDED BY IMPLICATION OR OTHERWISE AS IN ANY MANNER LICENSING THE HOLDER OR ANY OTHER PERSON OR CORPORATION, OR CONVEYING ANY RIGHTS OR PERMISSION TO MANUFACTURE, USE OR SELL ANY PATENTED INVENTION THAT MAY IN ANY WAY BE RELATED THERETO.

Reproduced by
DOCUMENT SERVICE CENTER
KNOTT BUILDING, DAYTON, 2, OHIO

UNCLASSIFIED